PROFILE OF HEMATOLOGICAL ABNORMALITIES AND ITS CORRELATION WITH ABSOLUTE CD4 COUNT AND HIV VIRAL LOAD IN HIV INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI

In fulfilment of the regulations for the award of the degree of

Doctor of Medicine in General Medicine

DEPARTMENT OF GENERAL MEDICINE

P.S.G INSTITUTE OF MEDICAL SCIENCES & RESEARCH

THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY,

CHENNAI, TAMIL NADU

APRIL 2016

PROFILE OF HEMATOLOGICAL ABNORMALITIES AND ITS CORRELATION WITH ABSOLUTE CD4 COUNT AND HIV VIRAL LOAD IN HIV INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In fulfilment of the requirements for the award of the degree of

Doctor of Medicine in General Medicine

Under the guidance of

PROFESSOR T.SARAVANAN, M.D.,

DEPARTMENT OF GENERAL MEDICINE

P.S.G INSTITUTE OF MEDICAL SCIENCES & RESEARCH, COIMBATORE

THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY,

CHENNAI, TAMIL NADU

APRIL 2016

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled, "**PROFILE OF HEMATOLOGICAL ABNORMALITIES AND ITS CORRELATION WITH ABSOLUTE CD4 COUNT AND HIV VIRAL LOAD IN HIV INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL"** is the bonafide original work of **Dr.S.SUJA** in fulfilment of the requirements for the degree of Doctor of Medicine in General Medicine.

Signature of the guide

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ENDORSEMENT BY THE HOD, DEAN / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled, "**PROFILE OF HEMATOLOGICAL ABNORMALITIES AND ITS CORRELATION WITH ABSOLUTE CD4 COUNT AND HIV VIRAL LOAD IN HIV INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL"** is the bonafide original research work of **Dr.S.SUJA** under the guidance of **Dr. T. SARAVANAN, M.D.**, Professor of Medicine, PSG IMS&R, Coimbatore in partial fulfilment of the requirements for the degree of Doctor of Medicine in General Medicine.

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**PROFILE OF HEMATOLOGICAL ABNORMALITIES AND ITS CORRELATION WITH ABSOLUTE CD4 COUNT AND HIV VIRAL LOAD IN HIV INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. T. SARAVANAN, M.D.,** Professor of Medicine, PSG IMS&R, Coimbatore.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University in fulfilment of the University regulations for the award of MD degree in General Medicine. This dissertation has not been submitted for award of any other degree or diploma.

Signature of the Candidate

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June 27, 2014

To
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Postgraduate
Department of General Medicine
PSG IMS & R
Coimbatore

Ref.: Proposal titled: *"Profile of hematological abnormalities and its correlation with absolute CD4 count and HIV viral load in HIV infected patients in a tertiary care hospital"*

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 10th June, 2014 in its full board review meeting held at Research Conference Room, PSG IMS&R, between 9.30 am and 12.30 pm, and discussed your application to conduct the study entitled:

"Profile of hematological abnormalities and its correlation with absolute CD4 count and HIV viral load in HIV infected patients in a tertiary care hospital"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed consent forms
4. Data collection tool
5. CV
6. Budget

The members who attended the meeting at which your study proposal was discussed are as follows:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
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3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	Yes
4	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	No
5	Mr. R. Nandakumar (Vice-Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
6	Dr. G. Rajendiran	DM	Clinician (Cardiology)	Male	Yes	Yes
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9	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Opthalmology)	Male	No	Yes
10	Dr. Seetha Panicker	MD	Clinician (Obstetrics & Gyanaecology)	Female	Yes	No
11	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
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14	Mrs. K. Uma Maheswari	M Sc, M Phil. B Ed	Botany	Female	No	No
15	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

After due consideration, the committee has decided to approve the above proposal.

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

We hereby confirm that neither you nor any of your study team members have participated in the voting/ decision making procedure of the committee. The members of the committee who have participated in the voting/ decision making procedure of the committee do not have any conflict of interest in the referenced study.

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22.6.17

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Member - Secretary
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22nd September,2014

Dear Dr Suja,

Greetings to you from Principal's Office!

Thank you for applying for the PSG **P**romoting **R**esearch **I**nitiative in **ME**dicine (**PRIME**), a notable initiative by the Research Office of the PSG Institute of Medical Sciences and Research, with the kind and generous support of PSG Management.

Your project *"Profile of hematological abnormalities and its correlation with absolute CD4 count and HIV viral load in HIV infected patients in a tertiary care hospital"* was blinded and sent for internal review.

Based on scores (Reviewer + Research credentials for Class A & B, and reviewer only for other classes), the Research Council, after a careful scrutiny of the scoring system and related procedures, is pleased to announce that your project has been selected for PRIME Grant at Class D level.

Please do sign at the copy of this letter to mark your acceptance of the same. Once that is received at the Principal's Office, you will receive further instructions on the Grant Utilization.

Dr S Ramaligam
Pricipal

To:
Dr Suja S
Ist Year, PG (MD General Medicine)
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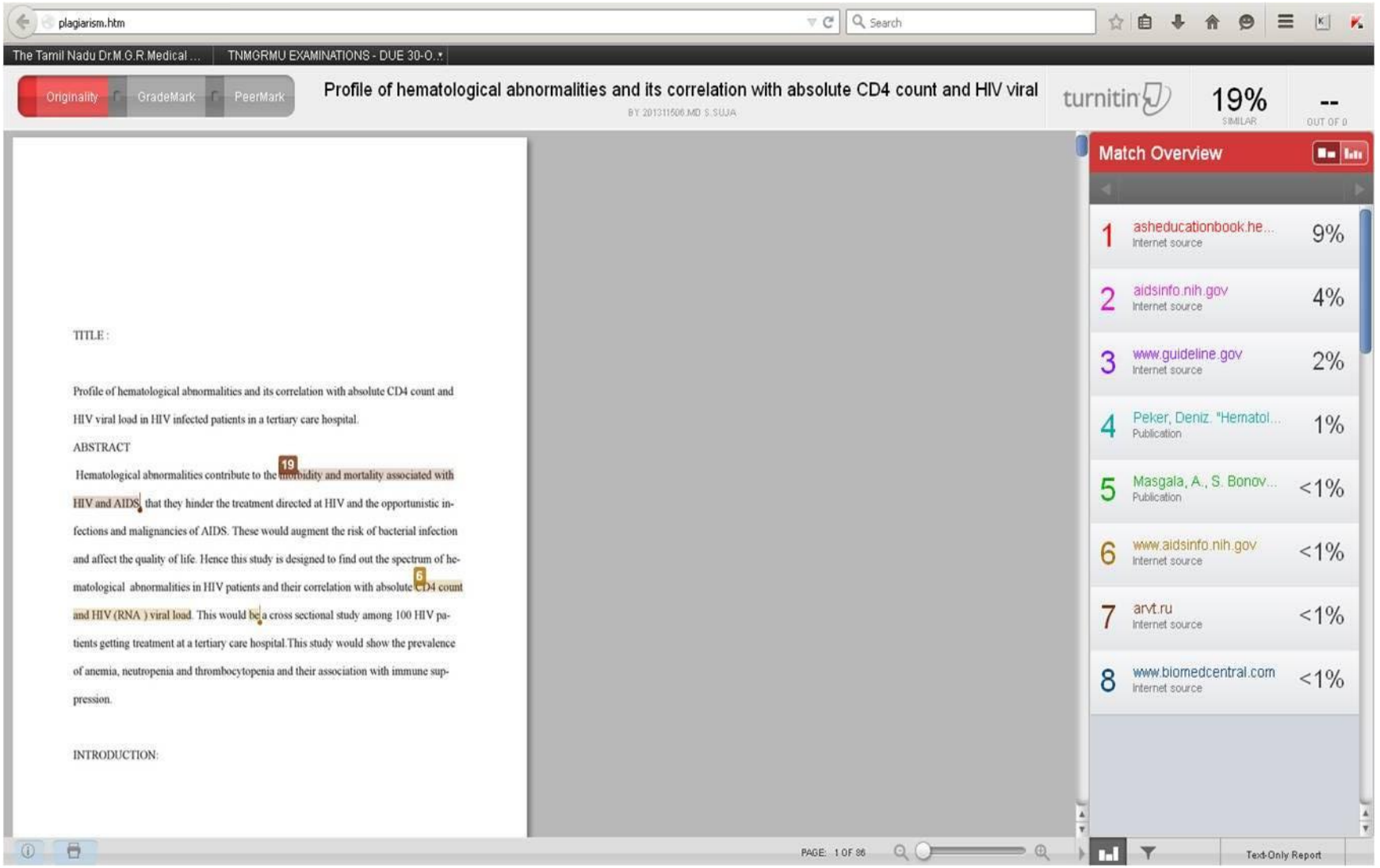
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Profile of hematological abnormalities and its correlation with absolute CD4 count and HIV viral load in HIV infected patients in a tertiary care hospital.
ABSTRACT
Hematological abnormalities contribute to the morbidity and mortality associated with HIV and AIDS, that they hinder the treatment directed at HIV and the opportunistic infections and malignancies of AIDS. These would augment the risk of bacterial infection and affect the quality of life. Hence this study is designed to find out the spectrum of hematological abnormalities in HIV patients and their correlation with absolute CD4 count and HIV (RNA) viral load. This would be a cross sectional study among 100 HIV patients getting treatment at a tertiary care hospital.This study would show the prevalence of anemia, neutropenia and thrombocytopenia and their association with immune suppression.
INTRODUCTION:
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ACKNOWLEDGEMENT

I would like to take this opportunity to express my gratitude towards Dr.T. Saravanan M.D, Professor of medicine, Department of General Medicine, P.S.G Institute of Medical Sciences & Research, Coimbatore, for his invaluable guidance and constant encouragement. His support and constructive suggestions were of immense help to me. I thank him for the expert supervision and unyielding patience in completing this work.

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My sincere thanks to the staff of Department of General Medicine, for helping me to carry out the study. Finally, I am very thankful and grateful to all those patients who were the subjects for this study, without whose contribution this work would not have been possible.

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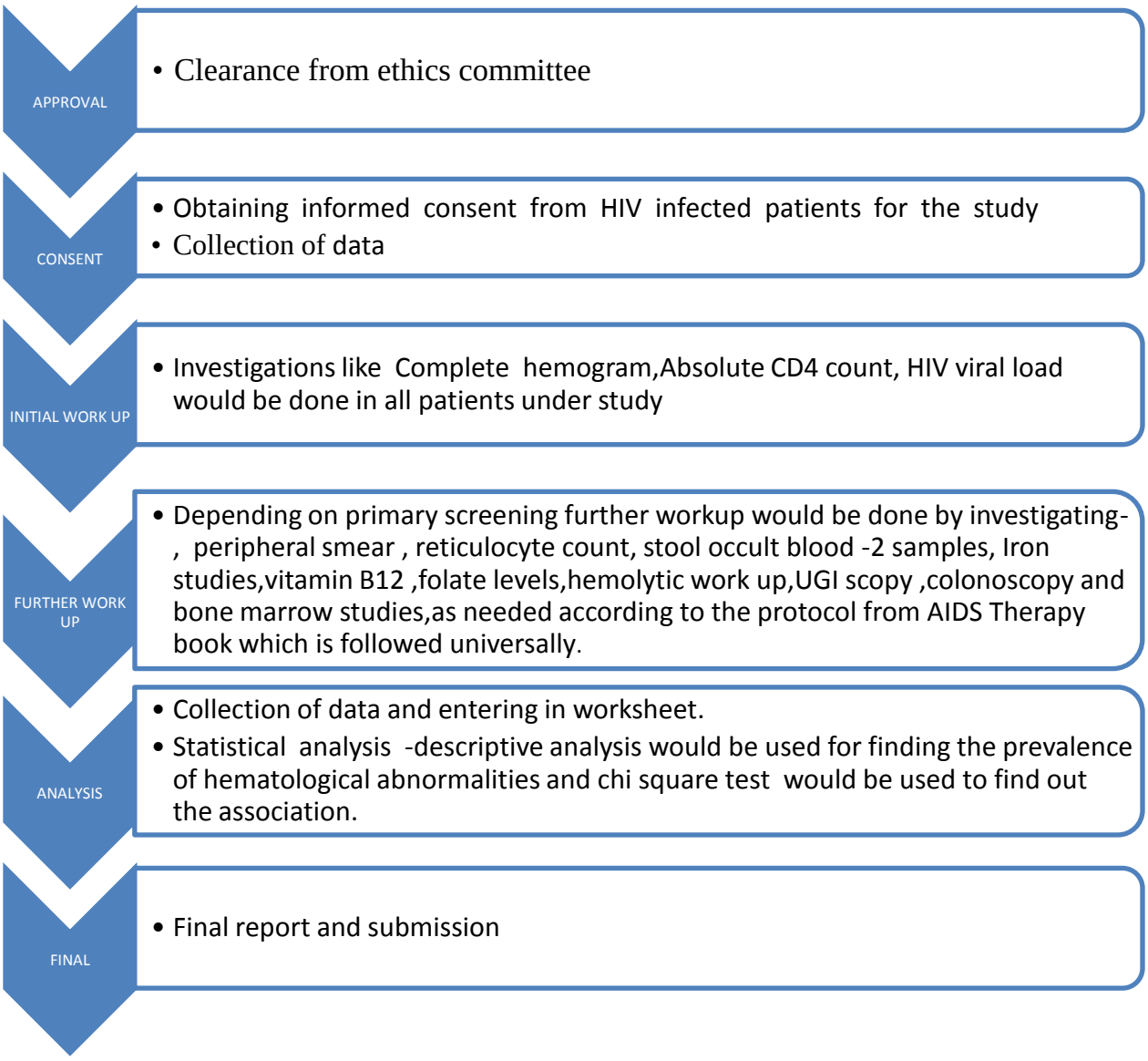
TITLE

PROFILE OF HEMATOLOGICAL ABNORMALITIES AND ITS CORRELATION WITH ABSOLUTE CD4 COUNT AND HIV VIRAL LOAD IN HIV INFECTED PATIENTS IN A TERTIARY CARE HOSPITAL

PROFORMA:


APPROVAL
• Clearance from ethics committee
CONSENT
• Obtaining informed consent from HIV infected patients for the study
• Collection of data
INITIAL WORK UP
• Investigations like Complete hemogram,Absolute CD4 count, HIV viral load would be done in all patients under study
FURTHER WORK UP
• Depending on primary screening further workup would be done by investigating- , peripheral smear , reticulocyte count, stool occult blood -2 samples, Iron studies,vitamin B12 ,folate levels,hemolytic work up,UGI scopy ,colonoscopy and bone marrow studies,as needed according to the protocol from AIDS Therapy book which is followed universally.
ANALYSIS
• Collection of data and entering in worksheet.
• Statistical analysis -descriptive analysis would be used for finding the prevalence of hematological abnormalities and chi square test would be used to find out the association.
FINAL
• Final report and submission

ABBREVIATIONS

AIDS	:	Acquired immunodeficiency syndrome
HIV	:	Human immunodeficiency virus
CD 4	:	Cluster of differentiation 4
PCP	:	Pneumocystis carini pneumoniae
HSV	:	Herpes simplex virus
RNA	:	Ribonucleic acid
ART	:	Antiretroviral therapy
DIC	:	Disseminated intravascular coagulation
HAART:		Highly active antiretroviral therapy
RBC	:	Red blood cell
DNA	:	Deoxyribonucleic acid
HBV	:	Hepatitis B virus
HCV	:	Hepatitis C virus
TB	:	Tuberculosis

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TABLE 5: PLATELET COUNT VS ABSOLUTE CD4 COUNT

TABLE 6: PLATELET COUNT VS ABSOLUTE CD4 COUNT (in treatment naive patients)

TABLE 7: PLATELET VS HIV VIRAL LOAD

TABLE 8: PLATELET COUNT VS HIV VIRAL LOAD (in treatment naive patient)

TABLE 9: ABSOLUTE NEUTROPHIL COUNT VS ABSOLUTE CD4 COUNT

TABLE 10: ABSOLUTE NEUTROPHIL COUNT VS ABSOLUTE CD4 COUNT (In treatment naive patient)

TABLE 11: ABSOLUTE NEUTROPHIL COUNT VS HIV VIRAL LOAD

TABLE 12: ABSOLUTE NEUTROPHIL COUNT VS HIV VIRAL LOAD (in treatment naive patient)

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FIGURE 14: ABSOLUTE CD4 COUNT VS COINFECTION

FIGURE 15: HIV VIRAL LOAD VS CO INFECTION

FIGURE 16: CO INFECTIONS

INTRODUCTION

Hematological abnormalities contribute to the morbidity and mortality associated with HIV and AIDS, that they hinder the treatment directed at HIV and the opportunistic infections and malignancies of AIDS. These would augment the risk of bacterial infection and affect the quality of life. Hence this study is designed to find out the spectrum of hematological abnormalities in HIV patients and their correlation with absolute CD4 count and HIV (RNA) viral load. This would be a cross sectional study among 100 HIV patients getting treatment at a tertiary care hospital. This study would show the prevalence of anemia, neutropenia and thrombocytopenia and their association with immune suppression.

A variety of hematological manifestations are seen at every stage of human immunodeficiency virus infection /acquired immunodeficiency syndrome and they often pose a great challenge in the comprehensive management. Hematological abnormalities and their complications are the most common cause of mortality and morbidity in HIV infected individuals.

Anaemia is the commonest hematological abnormality associated with HIV infection and anaemia is present in about 10-20% HIV infected patients at diagnosis, and their prevalence can range from 66-85% during disease course.Neutropenia occurs in 10-30% of individuals affected with HIV infection, typically in those with advanced disease stages. Thrombocytopenia has

been seen in 3-40% of individuals and it may be the initial presentation, and can occur at any stage of the disease.

Anemia and neutropenia are mostly because of inadequate production due to suppression of bone marrow by HIV infection .This occurs because of abnormal cytokine expression and alteration in bone marrow micro environment.Whereas thrombocytopenia occurs by immune-mediated destruction of the platelets, in addition to inadequate platelet production. Other causes of cytopenia in these patients include treatment-related adverse events or secondary to the opportunistic infections or neoplasms, or preexisting or coexisting medical issues. HIV infected patients with cytopenia require bone marrow examination to determine the cause of cytopenia and also to direct appropriate therapy.

The severity of cytopenia and their incidence are usually correlated to the stage of the disease. These manifestations also reflect the underlying immune status if interpreted cautiously, especially if the patient is in regular follow-up.

Hematological abnormalities can be the initial presentation of HIV infection. Patients may be asymptomatic, because of abnormal blood counts or lymphoid disorders, usually they were referred. By considering the possibility of HIV infection, have the opportunity to diagnose and treat the patients earlier and prevent transmission of the infection. Hematological abnormalities may be the direct result of HIV infection or manifestations of secondary infections, neop-

lasms or side effects of the therapy. It is necessary to identify and treat for hematological abnormalities to reduce the morbidity and mortality.

Though many studies have been conducted in India on HIV manifestations, in most of them, various aspects were addressed and the focus on the hematological manifestations were limited. Most of the available data is from the west, which might not be directly applicable to our Indian population. This study was planned, since there is paucity of information from Indian population regarding the hematological manifestation.

AIM

Primary objective:

To study the spectrum of hematological abnormalities in HIV infected patients

Secondary objective:

To find the correlation of hematological abnormalities with absolute CD4 count and HIV viral load

MATERIALS AND METHODS

INCLUSION CRITERIA:

- Age above 18 years
- HIV infection confirmed by western blot or ELISA method
- Patient giving consent for the study
- Patients as both inpatient or outpatient

EXCLUSION CRITERIA:

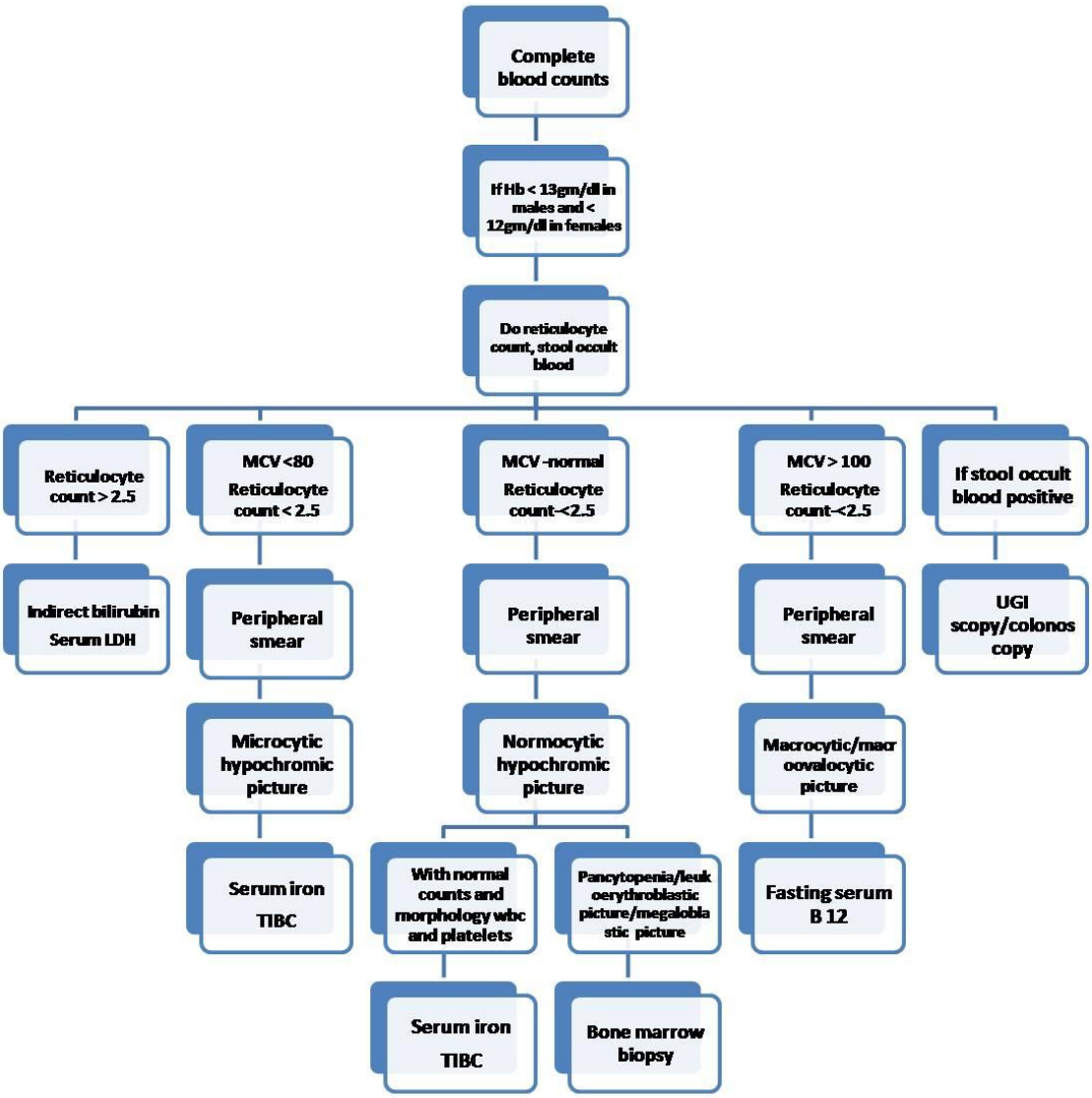
- Patient refusing consent
- Incomplete investigations

STUDY DESIGN: Observational study

STUDY LOCATION: PSG Hospitals, Coimbatore.

SAMPLE SIZE: 100

The hematological protocol followed in the study is given below:



REVIEW OF LITERATURE

HIV viral load and absolute CD 4 count are the most essential biomarkers correlating to the stage of HIV disease and its progression (1)(2).These tests though they are important biomarkers , they are costlier . Hence there is difficulty in doing these tests in HIV infected patients in developing countries .In HIV patients cytopenias are commonly noted (3)(4).Various hematological abnormalities are noted in every stage of HIV infection and they are of big challenge for effective management .

For the sake of economical evaluation of the status of HIV disease stage and its progression, complete blood counts and peripheral smear has been suggested as the alternatives(5). It is found that low haemoglobin count is most common among HIV patients and it is the most common finding among patients on HAART therapy (6)(7) . Low haemoglobin levels were also found to correlate with increased severity of the disease and poor prognosis (8)(5)(9)(10).Normocytic normochromic anaemia is the common finding among the blood picture of HIV patients. (11) Reasons for anaemia was found to be a) decreased production because of associated infections ,ART drugs ,low erythropoietin levels or HIV infection per se ; b) RBC lysis due to autoimmune hemolytic anaemia ,DIC or thrombotic microangiopathy ; c) Nutritional deficiencies (12) . Neutropenia is also found in majority of patients with high viral load and low CD4 count (13) .Thrombocytopenia were noted during early stages of the disease and it was not associated with poor prognosis. Thrombocyto-

penia in HIV infection is due to immune complex mediated peripheral destruction and antiplatelet antibodies. Abnormalities in the bone marrow are noted at various stages of the HIV disease and more during later stage of the disease.HIV infection in the mesenchymal stem cells of marrow leads on to the various bone marrow abnormalities (14). HIV infection leads on to a histiocytic reaction and further resulting in hemophagocytic syndrome .This causes severe pancytopenia.HIV also causes decrease in progenitor cells in the bone marrow (15).

As per Paradela A et al study, they have noted bone marrowdefects in HIV infected individuals mainly showing features of increased cell counts, granulomatous changes and some amount of dysplasias (16). Studies conducted in vitro has shown direct influence of HIV on mesenchymal cells and hematopoietic cell activity (14).

Cytopenias are the leading cause of morbidity and mortality in HIV patients. Studies done world-wide says, the prevalence of anaemia in HIV infected persons has been found to be as high as 63% to 95%, they also say that anaemia is more common than thrombocytopenia or leukopenia . In a US based study, Mildvan et al noted the prevalence of mainly anaemia in 9690 HIV infected individuals, and showed 39.5% (1721) of patient receiving no antiretroviral therapy and 35.5% (7252) of patients receiving HAART were anemic. They found anemia was more prevalent among men and patients with CD4<200 cells/mm3 (17). Hematological manifestations also reflect the underlying im-

mune status if interpreted cautiously, especially if the patient is in regular follow-up. Volberding et al reported that more severe degree of anaemia were found among HIV infected individuals presenting with low CD4 counts (18)

Among leucopenia, neutropenia is more prevalent in HIV patients. Its prevalence is upto 10-30% at later stages of the disease. HIV infection leads to bone marrow depression causing decreased granulocyte colony-stimulating factor levels, thereby causing low granulocyte-macrophage lineage. Thuscausing leukopenia and neutropenia in HIV patients. Bone marrow suppressive drugs or opportunistic infections may cause leucopenia. Moreover, HIV infection per se results in lymphopenia at later stages of disease, causing low CD4+ lymphocytes.

Various studies from developing nations showed a marked decline in mortality in HIV Patients following HAART therapy. An Indian study showed a dramatic decline in mortality from 25 to 5 deaths per 100 person years in HIV patients between 1997 and 2003 after starting HAART therapy (19). Factors like older age, baseline low CD4 counts, tuberculosis infection at any point of time and ART naive status has significant association with mortality. Higher mortality is seen in older age group, mainly above 35 years of age.Studies shows age as a poor prognostic factor. It was associated with lower CD4 counts which highlights poor prognosis in patients at later stages of the disease. A Brazilian study showed 5 times higher chances of death in patients who were symptomatic at entry than those who were asymptomatic.(20) Similar findings were noted in

an Jamaican study (21) and in an Indian study on ART naive patients on HAART therapy (22).A Sub-Saharan African study reported 8 – 26% mortality which is higher than the Taiwan study which showed mortality of 10.2 per 100 person years. Collaboration of prospective studies from Europe and United States showed that ART halved the mortality rate in HIV patients (23). There was another Sub-Saharan African study which reported early mortality in patients accessing HAART programmes and this was because of late initiation of HAART and associated opportunistic infections like tuberculosis, cryptococcal meningitis, malignancy and wasting syndrome (24). These studies emphasizes the need for creating awareness among HIV patients and their health care providers about early diagnosis of the opportunistic infections and timely initiation of HAART before advanced immune suppression occurs to reduce the morbidity and mortality. Indian studies showed tuberculosis as the commonest among opportunistic infections seen in HIV patients who died (22)(25)(26). This was the basis for, early detection and treatment of HIV as well as TB in the National AIDS Control Program of India (NACP). Recently,national guidelines in India recommends initiation of anti tubercular treatment (ATT) in HIV-TB co-infected individuals with lower CD4 count (350 cells/µl). It is recommended to start antiretroviral drugs in these patients after 2 weeks of initiation of ATT. A Brazilian study showed dramatic decline in the incidence of tuberculosis when HIV diagnosis and treatment were timely done (27).Reports says

that early HAART ,that is starting within two months of ATT is favorable compared to late and deferred HAART in reducing mortality (28) .

Studies says that at some point of course of HIV illness anemia can be found in 63%–95% of affected individuals. In the Multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project, Sullivan and colleagues,analysed cytopenia in 32,867 persons affected with HIV infection. They showed anemia occurrence increases with the clinical severity of the disease (29). 3% among HIV patients developed anaemia at 1 year follow-up, compared to 12% among those who had immunologic AIDS that is CD4 count < 200/μL and 37% among those with clinical AIDS (32). Among cytopenia, anaemia in an HIV-infected individual is significantly correlated with higher mortality, and it is independent of the absolute CD4 count and HIV viral load (8). But in converse, decreased risk of death has been noted in those who recovered from anaemia. Most common reason for anaemia in HIV patients is the anaemia of chronic disease. Very low level of CD 4 and CD 11a, expressed by CD34+ stem cells, leads them to be resistant to direct viral infection. The mononuclear macrophage cells develops productive HIV infection and the results in the release of cytokines, such as transforming growth factor-beta (TGF-β), interleukin 1, tumor necrosis factor-alpha (TNF-α).These cytokines leads on to the suppression of hematopoiesis.Though the levels of serum erythropoietin may be high in these patients, the response to anaemia is blunted compared to those with uncomplicated iron deficiency anaemia of same severity (30).

Drug treatment and the subsequent complications also the common reasons for anaemia among HIV patients. Zidovudine is the most common among the ART drug to cause anaemia. It inhibits the in vitro erythroid colony formation. It does it in a dose dependent manner (31). It is severe anemia, when hemoglobin level of less than 7.5–8.0 g/dl or anemia that is requiring transfusion, is noted in 24% of those who receive zidovudine 1.5 g /day. Following zidovudine intake, macrocytosis develops within weeks. Macrocytosis in these patients also acts as a useful marker of drug compliance. But neither vitamin B12 or folic acid will help in preventing zidovudine indced bone marrow toxicity. It is noted that serum vitamin B12 levels were often low in HIV-infected patients. But only a very few patients will have true vitamin B12 deficiency. It is diagnosed by elevated levels of homocysteine and also methylmalonic acid (32).

Human parvovirus B19, DNA virus, which is a single stranded small virus cause chronic and severe anemia in HIV patients. This DNA virus affects through respiratory tract .This virus reaches erythroid cells by P antigen in the blood group. It multiplies ellaborately, ultimately leading onto lysis of the infected cells. Due to the poor humoral immunity in late stages of HIV infection, the host response of generating neutralizing immunoglobulin IgM or IgG antibodies become reduced. Hence viremia remains uncontrolled, resulting in absent red cell production, with an arrest in maturation of erythroid cell lineage. Characteristically the giant cells like pronormoblasts were seen in bone marrow smears. But these characteristics are not that common, so bone marrow aspira-

tion is not required for diagnosing parvovirus B19. Alternatively polymerase chain reaction assays can be done to diagnose the parvo virus infection. One of the study done in University of Washington showed occurrence of this infection in about 17 percent of HIV patients with anaemia.This was done by dot blot hybridization (33) .Treatment includes intravenous immunoglobulin (IV IG) therapy about 400 mg/kg/day for 5 days to give antibodies to clear off the infection.This was not successful in many patients (34). There are case reports suggesting usage of very active antiretroviral therapy followed by subsequent reconstitution of humoral immunity, will lead to the recovery of parvovirus B19 infection (35).

CAUSES OF ANAEMIA:

DECREASED PRODUCTION

DRUGS

- Zidovudine
- Trimethoprim-sulfamethoxazole
- Amphotericin B
- Ganciclovir
- Dapsone
- Delavirdine

Deficiencies

- Erythropoietin

- Iron
- Folate
- Vitamin B12

Infection

- HIV
- Parvovirus B19
- Mycobacterium avium complex (MAC)
- Mycobacterium tuberculosis
- Histoplasma capsulatum

Neoplasia

- Non-Hodgkin’s lymphoma
- Multiple myeloma
- Castleman’s disease
- Hodgkin’s disease
- Miscellaneous
- Anemia of chronic disease
- Preexisting condition (sickle cell disease, thalassemia)

INCREASED LOSS

HEMOLYSIS

- Thrombotic thrombocytopenic purpura
- Glucose-6-phosphate dehydrogenase deficiency
- (trimethoprim-sulfamethoxazole [TMP-SMX], dapsone,
- primaquine)
- Autoimmune hemolytic anemia

IDIOPATHIC

DRUGS

- Ceftriaxone, indinavir, “Ecstasy”

INFECTION

- Cytomegalovirus [CMV]

GASTROINTESTINAL BLEEDING

- Kaposi’s sarcoma
- Non-Hodgkin’s lymphoma
- Infection (CMV, Candida)

HYPERSPLENISM

- Infection
- Lymphoma
- Hemophagocytosis
- Cirrhosis (hepatitis B virus [HBV], hepatitis C virus [HCV])

Mycobacterium avium complex (MAC), Mycobacterium tuberculosis (MTB), and Histoplasma capsulatum cause anemia in AIDS patients by bone marrow infiltration. Patients from Southeast Asia and southern China may also develop anemia due to Penicillium marneffei infection .Bone marrow aspiration and biopsy showed the evidence of infection in about 25%–42% of patients tested .However, evidence of infection can be made equally faster and accurately using less-invasive modalities like blood culture, serology, or nucleic acid hybridization. Especially true in case of mycobacterial infection. Bone marrow results in unique diagnosis, only in about 10% of the performed procedures, and is more likely to be helpful in patients with low CD4 counts or a hematocrit less than 25%. Surprisingly, autoimmune hemolytic anemia (AIHA) is not that common among HIV infected patients. Though the prevalence of a positive direct anti globulin test (DAT) ranges from 18% to 43% in this population, only few case reports about HIV-associated AIHA are there. Generally DAT-positive patients have lower hemoglobin levels than DAT-negative patients. This is because of the prevalence of DAT-positivity. Among the few cases of symptomatic autoimmune hemolytic anemia reported in HIV infected patients, both warm and cold antibodies have been noted, simultaneously. Reticulocytopenia is relatively frequent among HIV infected individuals hence its presence cannot be used to rule out hemolysis. But they show decreased haptoglobin level and elevated lactate dehydrogenase level, and bone marrow will be showing normocellular with erythroid hyperplasia. Treatments with corticosteroids,

intravenous immunoglobulin, withdrawal of any offending drugs, and splenectomy has shown success (36). Aggressive transfusion therapy should be handled with extra caution in HIV-associated AIHA, because of fatal pulmonary embolization due to increased hemolysis and disseminated intravascular coagulation which has been documented (37). Indinavir, ceftriaxone, and "Ecstasy" have been reported to cause hemolytic anemia in AIDS patients. Rarely, cytomegalovirus (CMV) infection also can cause hemolysis. Easy fatiguability is the main symptom of anemia, is also the most common symptom of HIV infection and is responsible for impaired physical function and poor quality of life. Many of the randomized, double-blind and placebo-controlled trials using a variety of regimens have noted that the use of recombinant human erythropoietin in HIV-associated anemia frequently augments the hemoglobin levels, fewer transfusions and a better quality of life.

One of the observational study done at Johns Hopkins hospital under HIV Clinic, showed among treatment of anaemic patients with erythropoietin resulted in a significantly decreased mortality. But in contrast, many studies suggested that transfusion therapy, early in the course of HIV infection, has been associated with increased mortality. Reason for this includes the transfusion-associated infection with agents such as CMV, hepatitis B and C, human T-cell lymphotropic virus (HTLV I/II) or parvovirus B19,transient activation of HIV expression; and transfusion-associated immune suppression which is mediated

by inflammatory cytokines that cause decreased lymphocyte, NK cell, and monocyte function.

Randomized trials done to compare the use of leuko reduced against unmodified red blood cells in patients with advanced stage of infection showed no evidence of cytokine activation post transfusion. Moreover, leuko reduction does not give any clinical benefit and have even lessen the survival. More number of transfusions can lead onto iron overload. This may aggravate progression of HIV and increases the susceptibility to infection by reducing macrophage function .Infections like oesophageal Candidiasis, Pneumocystis jiroveci and mycobacterium TB can occur.

HIV WITH THROMBOCYTOPENIA

Human immunodeficiency virus which is a retrovirus affecting immunity, impairing their immune function, thus leads on to the occurrence of opportunistic infections and malignancies. Hallmark of HIV infection is decreasing activity and number of CD4+ T lymphocytes and it also impedes other cell lineages and tissues. Cytopenias have been noted even without HAART or opportunistic infections and malignancies, proves that HIV infection is directly associated in causing these hematological derangements. Interestingly, thrombocytopenia is noted as the first clinical manifestation in otherwise asymptomatic HIV infected individuals. Neutropenia and anaemia are mostly noted in the later stages of HIV disease .Thrombocytopenia is identified if platelet counts are below

125000/mm3, and commonly occurs in HIV infected individuals. Possible mechanisms considered for thrombocytopenia are increased destruction of platelets, by immune complexes in circulation which are nonspecific gets deposited on the platelets or by the existence of specific anti-platelet antibodies, as well as direct action on megakaryocytes by HIV per se causing ineffective platelet production. Thrombocytopenia incidence among HIV patients is about 40% and in 10% of HIV patients, it may be the first sign of AIDS. Mature Megakaryoctes (MKs) gets affected by the HIV through CD4 receptor binding, and HIV genomes were detected in MKs that were purified from the bone marrow. MKs are affected by both X4- and R5-tropic HIV-1strains thus representing thrombocytopenia occuring in initial period of HIV infection. In earlier stages of the disease, in addition to the direct effects on MK cell lines, HIV also leads on to chronic thrombocytopenia by autoimmune mechanisms. Auto -immune mechanisms by molecular mimicry causing cross-reaction of anti-HIV antibodies against platelet membrane glycoproteins. Thrombocytopenia is related to increased morbidity and mortality, by accelerating the deterioration of CD4 counts and leading on to progression to AIDS.

It was in the year 1982, the association between HIV and thrombocytopenia has been reported for the first time (38).Now immune thrombocytopenic purpura (ITP) remains the most common cause of low platelet count in these patients . It is estimated about 30 percent occurrence (39) .ITP occurs in early stage of HIV infection because of platelet associated antibodies and occurs even before

other manifestations of the infection.HIV-associated ITP is commonly seen among men than women.

CAUSES OFTHROMBOCYTOPENIA

DECREASED PRODUCTION

Drugs

- Trimethoprim-sulfamethoxazole
- Pentamidine
- Pyrimethamine
- Ganciclovir
- Fluconazole
- Alpha-interferon
- Rifabutin
- Clarithromycin
- Didanosine
- Amphotericin B
- Indinavir
- Ritonavir
- Delavirdine
- Nelfinavir
- Deficiencies
- Folate
- Vitamin B12

Infection

- HIV
- Parvovirus B19
- Mycobacterium avium complex (MAC)
- Mycobacterium tuberculosis
- Histoplasma capsulatum
- Bartonella henselae (bacillary angiomatosis)

Neoplasia

- Non-Hodgkin's lymphoma

Miscellaneous

- Preexisting condition

INCREASED LOSS

- Immune thrombocytopenic purpura
- Thrombotic thrombocytopenic purpura
- Hypersplenism
- Infection
- Hemophagocytosis
- Cirrhosis

Drugs

- Saquinavir
- Interferon

Dominguez and coworkers noted platelet kinetics in 41 HIV patients with thrombocytopenia and found that the platelet survival was decreased in those with CD4 counts >200 Cells / μl than in those with counts <200, which shows that platelet destruction is more in patients with higher CD4 levels, and decreased platelet production is more among those with lower CD4 levels (40). Similar studies done by Cole et al showed that in HIV infected individuals there is ineffective delivery of viable platelets to the peripheral circulation, despite a 6-fold rise in thrombopoietin levels and a 3-fold expansion of megakaryocyte mass compared to the normal control groups (41). This gives the possibility of HIV-induced apoptosis of megakaryocytes and it correlates with the results of kinetic experiments, which showed increased platelet turnover but no change in platelet survival after zidovudine therapy. This indicates that the platelet production improves during treatment. Features of megakaryocytic infection by HIV virus includes denuded nuclei and ballooning of the peripheral zone of megakaryocytic cytoplasm which were observed by electron microscopy. Internalization of HIV particles were noted in co culture studies. The immuno histochemical techniques showed the presence of the HIV p24 antigen and HIV RNA expression noted using in situ hybridization.

Infections, malignancies or drugs can also lead onto impaired platelet production and thrombocytopenia, by bone marrow infiltration. About 8% of HIV patients have thrombocytopenia leading onto hemorrhagic event (42).Treatment is required only when platelet count is < 30,000/μl or if they are symptomatic.

Patients with coagulopathies and hemophilia must be requiring therapy when the platelet counts are < 50,000/µl , inview of very high bleeding risk..About 18% of patients with HIV-associated thrombocytopenia goes for spontaneous remission.

Specific treatment modalities for HIV-associated ITP includes corticosteroids,IVIG, intravenous anti-D therapy, splenectomy, danazol, interferon, and vincristine. Prednisone 1 mg/kg /day, increases the platelet count in many of the HIV patients but long-term use will result in Cushing's syndrome. It also accelerates the course of Kaposi's sarcoma and increases the risk of fungal infection .IVIG infusion induces quicker but unsustained remissions in about 71%–100% of HIV patients and it is also costlier (43).

Intravenous anti-D therapy increases the platelet count > 50,000/µl only in 34% of patients treated with it but it is less expensive. The response to anti-D is longer than that seen with IVIG therapy but the extent of hemolysis is unpredictable with D+ (Rh+). In patients with baseline hemoglobin levels > 12 g/dl are likely to have an elevation of their platelet counts than those with anemia.

Splenectomy is also found to be successful and, despite early concerns, does not obviously increase the risk of progression of HIV infection to symptomatic AIDS. A cohort study of 45 individuals and about 17 had splenectomy. In 28 individuals who did not have splenectomy, demonstrated a significant reduction in the risk of developing full-blown AIDS and showed reduced mortality

in splenectomized patients (44). This may be because of temporary reduction in plasma viremia and an increase in absolute CD4 and CD8 levels.

Thrombotic microangiopathy (TMA) is also a noted complication of HIV disease. It is seen in 1.4% of affected patients prior to the introduction of anti retroviral therapy (45).Both thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) were documented. When compared to TTP, HUS is more noted at advanced stages of HIV disease and found to be treatment refractory and results in death.

NEUTROPENIA

In HIV patients lower values of absolute neutrophil counts are because of granulopoiesis inhibition by the HIV virus itself or by bone marrow infiltration by infections .It can occur due to malignancies, adverse drug reactions, autoimmunity, and also due to hypersplenism.

CAUSES OF NEUTROPENIA:

Decreased Production

- Drugs
- Ganciclovir
- Zidovudine
- Trimethoprim-sulfamethoxazole
- Pentamidine
- Rifabutin
- Antineoplastic chemotherapy

- Dapsone
- Amphotericin B
- Ritonavir
- Delavirdine
- Nelfinavir
- Deficiencies
- Folate
- Vitamin B12
- Infection
- Human immunodeficiency virus (HIV)
- Mycobacterium avium complex (MAC)
- Mycobacterium tuberculosis
- Histoplasma capsulatum
- Neoplasia
- Non-Hodgkin’s lymphoma
- Multiple myeloma

Increased Loss

- Autoimmune neutropenia
- Hypersplenism
- Infection
- Hemophagocytosis
- Cirrhosis

Neutropenia in HIV patients is treated by initiation of antibiotic therapy for the evidence of infection; antiretroviral therapy for those who remain untreated for HIV infection; withdrawing of potential offending drugs; and myelopoiesis stimulation using either granulocyte colony stimulating factor (G-CSF) or granulocyte macrophage colony stimulating factor (GM-CSF).

THROMBOSIS

Thrombosis in HIV-infected patients has been reported up to 2%. Venous thromboembolic complications are associated with factors like age more than 45 years, advanced stage of HIV infection, the presence of AIDS-defining opportunistic infections,treatment with indinavir or megestrol acetate (46). Immobilisation due to illness is commonly behind the association between opportunistic infections and thrombosis (47). But in case of CMV infection, it promotes adhesion of platelets and neutrophils to the endothelium which will induce production of anti phospholipid antibodies.These enhance synthesis and increases the secretion and survival of factor VIII and von Willebrand factor (48). Reason for indinavir induced thrombosis is unclear, but megestrol, like many other progestational agents, leads to acquired resistance to activated protein C.

Moreover individuals with HIV infection will be at increased risk for thrombosis because of the various reasons like decreased levels of anti thrombin, protein C and S, or heparin cofactor II; coexistence of malignancy , autoimmune

or inflammatory disorders; the presence of anti cardiolipin antibodies or vascular damage due to injection drug use, intravenous catheter placement or CMV infection.

Anti thrombin deficiency occurs in association with HIV nephropathy as a result of loss of anti thrombin in the urine. The nephrotic syndrome which is seen in HIV nephropathy may be resulting in compensatory hepatic synthesis of factors V, VIII, and X induced by hypoalbuminemia and thereby increased platelet adhesion and aggregation (49). Acquired protein S deficiency is seen in upto 75% of HIV-infected children and adults. This is especially seen in patients with CD4 counts < 200/μl or AIDS and results in thrombotic complications in upto 12% (50). Free protein S antigen levels in HIV infected patients can be appearing artificially lower when assayed by PEG precipitation method, so that the prevalence of protein S deficiency in HIV patients may actually be lower than previously thought of about 10% (51). Lupus anticoagulant is noted in about 0- 70% of HIV patients, depending on the sensitivity of the assay used and the patients characteristics. Anti cardiolipin antibodies are found in 46-90% of the HIV patients. Neither in HIV is strongly associated with high risk of venous thromboembolic manifestations, but events such as multiple transient ischemic attacks ,stroke, skin necrosis, avascular necrosis of bone and brachial artery thrombosis have rarely been described in patients with anti cardiolipin antibodies (52).

Hemophagocytic Syndrome in HIV:

Hemophagocytic syndrome in HIV infected individuals is a rare association.Pancytopenia ,high grade fever,enlargement of the spleen and lymphadenopathy are the features.This syndrome has characteristic bone marrow histiocytosis and blood cell precursor phagocytosis.HIV infection per se can cause this syndrome or this can occur because of the association cytomegalovirus, HSV,histoplasmosis,TB,human herpesvirus 8, and parvovirus B19 infections.This hemophagocytosis lead onto worsening of malignancies like non-Hodgkin's lymphoma and Kaposi's sarcoma (53).

VIRAL MARKERS

Surrogate markers of treatment responses in HIV patients are HIV viral level and absolute CD 4 T lymphocyte level. HIV RNA load is an important marker for assessing the treatment response .Viral load level prior to HAART and level of decrease after treating with drugs provides details regarding prognosis and about the possibility of progression of illness (54).Main target of ART is to attain a sustained very low viral level. Hence HIV viral level is used to look for the efficiency of ART following commencement. CD4 counts are measured during initiation of treatment with ART. It gives the estimate of overall immune status of HIV patient. Absolute CD4 count is very critical to establish the thresholds for the starting and discontinuing prophylaxis for opportunistic infections and in deciding the initiation of ART.

With the availability of more potent anti retroviral drugs management of HIV infection has changed substantially. In USA, ART is provided to all HIV infected individuals regardless of their immune status as assessed by HIV viral level and absolute CD4 cell count.

HIV -RNA VIRAL LEVEL:

Human immunodeficiency virus (HIV) infection is persistent and progressive in majority of untreated individuals .There is a strong proof that HIV specific cellular immune responses are the key in determining the viral replication tempo and their clinical course of the disease. The proof for this protective immune mechanism comes from the rhesus macaque model of SIV (simian immunodeficiency virus) infection. It shows (i) $CD8^+$T-cell function interference with monoclonal antibody mediated destruction increases replication of HIV and (ii) $CD8^+$ cytotoxic T lymphocytes (CTL) has the capacity to exert prime pressure on the HIV genome, and this is evident by faster appearing specific escape mutations in epitope-encoding sequences. Recently studies shows vaccines capable of exhibiting viral specific $CD8^+$ T-cell responses which controls HIV replication and prevention of disease occurrence (55).

The recent Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents given in the website

- http://aidsinfo.nih.gov/guidelinesare as follows

HIV-1 VIRAL LOAD ASSESSMENT:

Human immunodeficiency virus RNA is a good marker of early and sustained treatment response (AI) .Viral load has to be measured among all patients during their first visit (AIII), at the commencement of treatment (AIII), and during follow up. Those who are not opting treatment, viral load testing is an optional (CIII).HIV Viral load prior to treatment is necessary to decide which regimen of ART to be started. This choice of regimen is because present ART drugs show poor responses if there is a very high level of HIV viral load. HIV-1 RNA assays which are available currently does not detect HIV 2 viral load.

Many systematic analysis of data through clinical trials showed marked decrease in viral load following HAART (54,56). Therefore viral load testing is an important marker correlating to the treatment response in HIV patients. Observers noticed that 3 fold change in viral load that is approximately $\log_{10}$ copies/micro liter is statistically significant. If the viral load is consistently below the detection level <20 to 75 copies/ micro litre it is called Optimal viral suppression. However in few successfully treated HIV patients the viral levels remains temporarily lower about HIV RNA level < 400 copies/ micro litre and that is not predictive of so called virological failure (57). Furthermore, review on data regarding the association between sustained low levels of about HIV viral load < 200 copies /micro litre ,the virologic failure is bit controversial. There is one study which showed more chance of further failure at this viral load level but this association was not proved in other

studies. AIDS Clinical Trials Group (ACTG)which defines virological failure if viral load >200 copies/micro litre. This is the level that eliminates apparent viremia caused by viral load blips and variabilities in the assays. Those who are adherent to ART achieve their viral load suppression in 8-24 weeks of treatment and they do not tend to harbor resistant strains .Only very rarely in few HIV patients it takes longer time to achieve virological suppression.

RECOMMENDATIONS FOR VIRAL LEVEL ASSESSMENT:

Following ART commencement / treatment adjustment due to virological failure, HIV viral load has to be measured within 2 to 4 weeks after treatment initiation or modification but not later than 8 weeks (AIII). This is needed for knowing adequacy of virological response to treatment and regularity in drug intake. HIV viral level has to be repeated in 4 - 8 weeks interval till the viral load level falls less than the detection limit (BIII).

Patients with previously attained virological suppression or in those with treatment change due to side effects or simplifying the treatment, HIV RNA level has to be done in 4 - 8 weeks following change of treatment regimen (AIII). This is to confirm that the new regimen is effectively bringing virological suppression or not.

In patients who are on a stable and effective ART regimen, HIV viral load has to be done in every three to four months (AIII) or else when needed to decide

maintained viral suppressive state. If patient is adherent to drugs and viral load is suppressed for nearly 2 years than clinicians can extend to every 6 months monitoring of viral load.

Patients who has less optimal response ,HIV viral level has to be monitored depending on the clinical circumstances, like adherence and if there is options for further treatment available.

CD4 COUNT MONITORING:

In HIV patients, absolute CD4 cell count acts as the main indicator of immunological status and acts as a marker of ensuing progression of illness and mortality .This is based on the clinical controlled trials and studies across the world.These counts are very much variable. Statistically significant difference among 2 tests is about a thirty percentage change in the absolute CD4 count, or a 3 percentage point increase or decrease in the CD4 percentage. Measuring lymphocyte subsets like CD8, CD19 other than CD4 has not found to be clinically useful. They are costlier and hence not suggested (BIII).

CD4 CELL COUNT FOR INITIAL JUDGEMENT:

Absolute CD4 cell count must be monitored in every patient at the reach of medical service (AI). CD4 counts are the important factor in deciding the need of initiation of opportunistic infection prophylaxis and the urgency in initiating ART (AI). Most of the opportunistic infections occur in patients whose CD4

counts were less than 200 cells/ cubic mm.Sometimes opportunistic infections were noted among patients with higher absolute CD4 cell level (58).

TREATMENT RESPONSE ASSESSMENT WITH ABSOLUTE CD4 CELL COUNT :

The immunologic response in HIV patients who were treated with ART is assessed with CD 4 cell counts .CD4 count is also used to decide whether prophylaxis for opportunistic infection can be discontinued or not. Mostly in patients on ART, CD4 cell count has to be raised upto 50 to 150 cells/ cubic mm during the first year of treatment.Then only it is said as adequate response.Mostly they show an increased response in the first three months of ART. Thereafter they have an average increase of upto 50 - 100 cells /mm^3 / year till reaching steady state in those who start treatment at a lower lymphocytes or those in old age.They usually have small increase in the cell counts even though they show virological suppressive state.

ABSOLUTE CD4 CELL COUNTS MONITORING:

Now treatment is recommended for all HIV positive patients. If patients not on treatment, CD4 counts has to be done every 3 to 6 months for assessing urgent need of ART commencement and starting opportunistic infection prophylaxis at right time (AIII).

Level of immune reconstitution can be assessed by measuring a repeat CD4 count after 3 months of ART initiation (AIII). This measurement is critical in

patients in whom ART is initiated at more advanced disease and in those requiring opportunistic infection prophylaxis or treatment. The time and extend for CD4 cells raise is needed to decide on continuation / stoppage of opportunistic infection prophylactic treatment. In situation like this and in the initial two years of treatment, CD4 cell count can be done in 3 to 6 months interval time (BII).

It is rarely indicated to change the drug regimen if there is poor CD4 response in an individual with good virological response. In those who have consistent suppression of viral loads and already attained ART-mediated immune reconstitution, level of CD4 count gives only little information. So frequent monitoring is unnecessary as it is not going to change the clinical management. A retrospective study showed that CD4 count of less than 200 cells/mm^3 are rare among HIV patients who has viral suppression and they usually had CD4 counts more than 300 cells/mm^3.The ARTEMIS trial has showed that CD4 testing had no benefit in patients who had viral load suppression and in those who had CD4 counts more than 200 cells/mm^3 after 48 weeks treatment with ART.

Studies found that in patients who have CD4 counts between 100 -200 cells/mm^3,the risk of Pneumocystis jirovecii pneumonia is very low who is on suppressive ART. Rarely in few patients with virological suppression,CD4 counts were very low and is associated with worst outcomes like ischemic heart disease, tumours and high mortality. In United States it is found that cost analysis of monitoring CD4 count every 12 months instead of 6 months could save

10 million dollars annually.Those who are on suppressive regimen with their CD4 count consistently found to be between 300 -500 cells/mm^3 for minimum of 2 years, then the Panel recommends monitoring of CD4 count can be done on an annual basis (BII). It is optional to monitor CD4 count in patients who has virological suppression and whose CD4 counts are consistently above 500 cells/mm^3 for minimum of 2 years (CIII). CD4 cell count must be done frequently, if clinically necessitated, if there are changes in the status clinically that would reduce CD4 cell count then CD4 count has to be monitored frequently and thus prompt opportunistic infection prophylaxis. Frequent monitoring is needed when changes like occurrence of new HIV related symptoms or commencement of drugs which cause reduction in CD4 cell count like corticosteroids or chemotherapeutic agents or interferon alpha(AIII). In patients with virological failure while adhering to treatment, CD4 cell count has to be done in every 3 to 6 months interval (AIII).

FACTORS THAT AFFECT ABSOLUTE CD4 COUNT:

On the basis of total leucocytes and total lymphocyte % and percentage of CD4 lymphocytes, value of CD4 count can be calculated. This absolute count may vary in individuals with the presence of acute infections and use of ART of which some are bone marrow-suppressive medications. In patients who have undergone splenectomy or co-infected with human T-lymphotropic virus type I (HTLV-1) there may be elevated CD4 counts, which would be misleading. Alpha-interferon therapy affects only the CD4 counts and not the percentage of

CD 4. CD 4 percentage remains stable in all these conditions and it may be the more appropriate marker to assess immune status of HIV patients.

DRUG-RESISTANCE TESTING

Testing for the drug resistance in HIV is suggested in all the patients during their first attendance to treatment centre. It has to be done for all patients for whom initiating antiretroviral therapy (ART) sooner or later (AII). Planned for ART later, then drug resistance test has to be done again at the initiation of ART regimen (CIII). In ART naive patients, genotypic testing is more preferred hence it is recommended (AIII). Standard method of genotypic testing of drug resistance includes testing for the RT (reverse transcriptase) and PR (protease) gene mutations. If RT and PR gene mutations are transmitted then INSTI (integrase strand transfer inhibitor) gene mutation has to be analysed (CIII). Drug-resistance testing is performed to decide on changing treatment regimen in those HIV patients with virological failure and in those with HIV viral load above1,000 copies/millilitre (AI). Even the drug resistance tests are unsuccessful among patients with HIV viral level above 500 but below 1000 copies/millilitre, testing is still to be considered (BII).

If there is suboptimal reduction in viral load ,then drug testing has to be considered (AII). Patients who fail from INSTI-based regimens, then INSTI resistance testing has to be done to know about adding drugs from this group (AII). If there is viral failure drug resistance tests has to be done when patient is on

the ARV medications otherwise within four weeks from discontinuing drugs (AII). When four weeks has lapsed from stoppage of drugs, still resistance testing gives useful information regarding the choice of treatment, but the previously selected gene mutations can be missed (CIII). While on first or second ART regimens genotypical tests are the resistance testing of choice, which guides treatment incase of virological failure (AII). The phenotypic drug testing is added to the genotypic testing generally for suspected or known mutation patterns mainly protease inhibitors (BIII).

For all pregnant women prior to initiation of ART, Genotypic drug resistance testing is recommended (AIII) and also for those who are already on ART with detectable viral load and entering pregnancy (AI).

CO-RECEPTOR TROPISM ASSAYS:

Panel recommendations are: Whenever considering treatment with CCR5 co-receptor antagonist co-receptor tropism assay has to be performed (AI). If not achieving viral load suppression with CCR-5 antagonist,then this tropism testing assay is recommended (BIII). For determining HIV-1 co-receptor usage a phenotypic tropism is the recommended choice of assay (AI).

Genotypical tropism assay can be used as an other test to analyse HIV 1 co-receptor usage (BII).

SCREENING FOR HLA B-5701 :

Before treating patients with abacavir (ABC) containing ART regimen, the Panel recommends to screen for HLA-B*5701 thereby the hypersensitivity reaction risk can be avoided (HSR) (AI). Those who are show HLA B-5701 must not be treated using ABC (AI). If HLA-B*5701 found positive then it has to be mentioned as ABC allergy in the medical records of the patient (AII). If HLA B -5701 screening is not possible, then ABC has to be initiated with proper counselling explaining the risk and has to be monitored for any signs of HSR (CIII).

Initiating Antiretroviral Therapy in Treatment-Naive Patients:

All HIV infected patients has to be treated with Anti retroviral therapy (ART) to decrease the risk of progression of the disease. Strength and evidence for this recommendation differs according to the pretreatment CD4 count level:

- CD4 count level less than 350 cells / cubic mm (AI)
- CD4 count level 350 - 500 cells/ cubic mm (AII)
- CD4 count level more than 500 cells / cubic mm (BIII).

ART is advised for HIV patients to cut down the HIV transmission. Strength and evidence of this recommendation is according to the risk of transmission like in perinatal transmission (AI), in hetero-sexual transmission (AI) and other risk groups mentioned (AIII).

Before commencing treatment patient has to be motivated for treatment adherence and commits to continue therapy and he must know the advantages of treatment and need for treatment adherence (AIII). Patient has the right to postpone therapy, and providers can opt to delay therapy on individual case by case basis, depending on clinical basis and psychosocial factors.

COMBINATION ART REGIMENS FOR INITIATING THERAPY FOR TREATMENT NAIVE PATIENT:

For a treatment-naive patient treatment has to be started with two NRTI (nucleoside reverse transcriptase inhibitors) and the third drug should be an active drug from following groups - an integrase - strand transfer inhibitor or NNRTI (non- nucleoside reverse transcriptase inhibitor) or PI (protease -inhibitor) .This regimen has to be given along with an enhancer like either cobicistat or else ritonavir.

DRUGS FOR TREATMENT NAIVE INDIVIDUALS:

Integrase Strand Transfer Inhibitor-Based Regimens:

- Lamivudine /abacavir or dolutegravir—this has to be used in those who are negative for HLA B -5701 (AI)
- Tenofovir/dolutegravir /emtricitabine (AI)

- Tenofovir/elvitegravir/cobicistat/emtricitabine— this has to be used only in those who have CrCl >70 mL/min prior to antiretroviral therapy (AI)
- Raltegravir / emtricitabine /tenofovir (AI)

Protease Inhibitor (PI)-Based Regimen:

- Tenofovir/emtricitabine plus darunavir/ritonavir (AI)

Emtricitabine and lamivudine can be exchanged.

Regimen to initiate the therapy should be guided by considering the following factors like virological efficacy, toxicity of the drugs,frequency of dosing, drug-drug interactions, results of resistance testing, comorbid illness, and the cost of the drugs.

MANAGEMENT OF THE TREATMENT-EXPERIENCED PATIENT:

Expert advice should be sought in case of virological failure. It is a complex process to assess and manage a patient experiencing antiretroviral therapy failure. In case of virologic failure adherence to drugs, interactions to drugs, treatment history, drug tolerability, HIV viral load and absolute CD4 cell counts and drug-resistance tests has to be looked for. If failure to antiretroviral (ARV) regimen is doubted drug resistance testing has to be performed (AI) in 4 weeks from discontinuation of drugs (AII). If 4 weeks after discontinuation also

though it may not be detecting previously selected resistance mutations, resistance testing can still provide useful information to guide the therapy (CIII).

In treatment experienced patients who has virologic failure with drug resistance,the goal of treatment is to attain virological suppression ,that is to bring-down HIV viral load below detectable limit (AI).

2 or 3 completely active drugs has to be given in newer combination (AI).Drug which is expected to have no drug resistance and with newer mechanism of action is considered to be entirely active pill.

Because of development of drug resistance, single drug should not be added to a failing regimen (BII).

In treatment experienced patients attaining good suppression is difficult (AI) hence regimens are designed to reduce toxicity and delay the clinical progression rate.

POORER CD4 COUNT RECOVERY AND SUSTAINED INFLAMMATION EVEN AFTER VIROLOGICAL SUPPRESSION

In HIV infected patients, complications from many AIDS and not pertaining to AIDS conditions are augmented even in those who are on antiretroviral treatment mediated virological suppression .These are predicted by sustained lower CD4 cell counts and consistent immune activation. Anti retroviral drugs should

not be added to suppressive regimen for augmenting the response since it does not improve CD4 cells hence it is not recommended (AI).

In those who attained virological suppression, switching over of ARV drug classes does not always improve CD4 cell counts or decrease immune activation and it is not recommended (BIII).

Presently interleukin-2 is not suggested to raise the CD 4 counts [AI] .None of these agents are proven to bringdown the severity while on ART mediated virological suppression.

Testing the immune activation markers and inflammatory markers are not suggested by the panel.Mostly markers predicting the severity shows fluctuating results among these patients (AII).

Since no methods to raise CD4 counts, only the modifiable risk factors has to be addressed like avoiding smoking, following healthy diet pattern and controlling hypertension and hypercholestrolemia (AII).

THERAPEUTIC DRUG MONITORING (TDM) FOR ANTIRETROVIRAL AGENTS

There is no recommendation about therapeutic drug monitoring of antiretroviral drugs for regular use in HIV patients management purpose (BII).

ANTIRETROVIRAL USE IN SPECIAL PATIENT POPULATIONS

ACUTE AND RECENT (EARLY) HIV INFECTION:

For all with HIV type 1 infection and with earlyHIV-1 infection antiretroviral therapy (ART) is recommended (BII), still strong information regarding its virological, immunological and clinical success are not available. Pregnant women in early stage of infection, the treatment should be commenced as early as possible to cut down perinatal transmission of HIV (AI). If treatment is started,the goal should be to bring down viral load to undetermined level (AIII).

If antiretroviral therapy is started in patients with early HIV-1 infection , check for the HIV-1 viral load, absolute CD4 cell counts as done in chronic HIV-1 infection (AII).

Before initiation of ART,the genotypical resistance testing of drugs should be done to choose the ART regimen (AII). Genotypic drug resistance has to be done even if the treatment is deferred for time being,since it would be useful in deciding the regimen for attaining optimal virological response if treatment is once commenced (AII).

If no drug resistance is found,then antiretroviral treatment has to be started . One of the combined regimen that is recommended for chronic HIV type 1 infection has to be used (AIII).

Antiretroviral treatment must be started before the resistance test are results are available for the drugs, since resistance to protease inhibitors developed in slower rate and also the resistance to protease inhibitors that are clinically significant is not that common. Protease inhibitors and two nucleoside reverse transcriptase inhibitors has to be used (AIII).

ART should be started in individuals who are committed to the treatment .They should be educated about the risks of treatment discontinuation and they must understand the importance of taking drugs regularly (AIII). These patients has the right to postpone the treatment until it is convenient for them .

WOMEN WITH HIV INFECTION:

Goal for starting of antiretroviral therapy in HIV-infected women are similar to other HIV infected adults and adolescent groups (AI). To prevent unintended pregnancy , those women who are on antiretroviral (ARV) drugs should use an additional or alternative contraceptive method, if their oral contraceptives have significant pharmacokinetic interactions with antiretroviral drugs.(AIII).

In pregnant women, HIV transmission perinatally should be prevented and it is an additional goal of therapy .The goal is effective virological suppression so that to bring down the risk of HIV transmission among the fetus and also in the newborn (AI). While selecting an antiretroviral regimen to use in pregnancy, physician must analyse the efficacy, other pharmacokinetic details and safety of each antiretroviral agent (AIII).

Before the initiation of efavirenz (EFV) childbearing aged women must check pregnancy tests and should receive information regarding the danger to the fetus and effective contraception methods to be taught while she on efavirenz based regimens (AIII). In case if they are planning pregnancy then efavirenz contained regimens should be totally avoided.

In efavirenz based regimen the risk of neural tube defects (NTD) are restricted to first 5 to 6 weeks of pregnancy but pregnancy is recognized rarely before 4 to 6 weeks of pregnancy.So that efavirenz can be continued in pregnant women who is receiving an efavirenz based regimen and presenting for antenatal care in the first trimester, provided the regimen resulting in virological suppression (CIII).

Mainly when designing a particular regimen for a pregnant woman, physician should always consult the most current Health and Human Services (HHS) Perinatal Guidelines (AIII).

SUMMARY OF HIV-2 INFECTION:

Generally HIV 2 infection disease course is marked by a long asymptomatic period ,compared to HIV type 1 infection .There will be usually lower HIV-2 RNA levels in the plasma and lower mortality rate but then progression to AIDS also occurs in HIV 2 infection .Antiretroviral treatment must be commenced prior to the occurrence of progression of the disease.

HIV-2 is usually resistant to the drugs like non nucleoside reverse transcriptase inhibitors and also resistant to enfuvirtide. Hence non nucleoside reverse transcriptase inhibitors and enfuvirtide should not be used in ART regimen for a HIV type 2 infected individual.

Definitive information about the outcome of antiretroviral therapy in a treatment naive patient with HIV-2 infection alone or HIV-1 and HIV-2 infection are pending.In these patients 2 nucleoside reverse transcriptase inhibitors and one HIV-2 infection active boosted protease inhibitor or one integrase strand transfer inhibitors must be commenced. But there are no validated tests to assess HIV-2 RNA load or absolute CD4 counts as done in case of HIV type 1 infection. But if there are no improvement in either clinical/virological /immunological status then an expert opinion is required.

HIV IN OLDER PATIENT:

Since there is increased risk of many complications that are not AIDS related ones and the reduced immunity to ART in older HIV infected patients,treatment with ART is recommended in all patients who are aged above 50 years with HIV infection, irrespective of their absolute CD4 count (BIII).

Older HIV infected individuals are more prone for ART-associated adverse events than younger HIV infected individuals.There is high chance of drug interactions among antiretroviral agents and other drugss which are routinely

used in elderly HIV infected patients. Hence they have to be monitored periodically, mostly while commencing ART or changing drugs.

HIV AND HEPATITIS B VIRUS CO INFECTION

Before starting ART,all HIV infected patients who are positive for hepatitis B surface antigen (HBsAg) should undergo hepatitis B virus DNA measurement by quantitative analysis .This finds out the estimation of HBV replication (AIII). Drugs like tenofovir ,emtricitabine and lamivudine will be having action against both HIV infection and HBV infection.Treatment for HBV orHIV if needed, then ART must be commenced with combination of TDF + FTC or with TDF + 3TC since there is nucleoside reverse transcriptase inhibitor (NRTI) backbone for a complete suppressive ART (AI).

When TDF cannot be used safely and HBV treatment is required then the alternative HBV therapy recommended is entecavir along with the completely suppressive ART combination (BI).Drugs for the treatment of HBV infection includes peg interferon alpha monotherapy or 3TC in combination with adefovir or telbivudine or else FTC along with the completely suppressive ART (BII).

Entecavir can be used along with the completely suppressive antiretroviral treatment regimen in HIV and HBV co infected individuals (AII), because entecavir when used without ART drugs for HBV infection,it leads to M184V mutation which will raise HIV resistance 3TC and also to FTC. Hence discontinuation of drugs with anti-HBV activity can cause very serious hepatocellular

injury resulting from reactivation of HBV.Thus patients should be advised against self-discontinuation and should be carefully monitored during interruptions in HBV treatment (AII).

If ART needs to be modified due to HIV virological failure and the patient has adequate HBV suppression, the ARV drugs active against HBV should be continued for HBV treatment in combination with other suitable ARV agents to achieve HIV suppression (AIII).

HIV AND HEPATITIS C VIRUS CO INFECTION

Every HIV infected individuals must be screened routinely for hepatitis C virus (HCV) infection. Individuals at risk must be screened once in a year and also screened whenever HCV infection is in doubt.

Antiretroviral treatment can slowdown the liver disease progression by maintaining the immune capacity and decreasing the HIV mediated immune activation and process of inflammation. In majority of the HIV and HCV-co infected patients, the benefit of antiretroviral therapy outweighs concerns regarding drug induced liver injury even in those with cirrhosis. Hence antiretroviral treatment should be started in HIV and HCV-co infected patients, irrespective of absolute CD4 cell counts (BII).

Antiretroviral treatment regimens advised for most HIV and HCV-coinfected patients are same as those recommended for individuals without HCV infection. But, when treatment for both the infection are indicated, the antiretrovir-

al treatment should be decided based on the potential drug interactions and their toxicities with HCV treatment combination.

Combined treatment of HIV and HCV will lead onto complications due to drug interactions. But still ART should be started in most HIV/HCV co infected patients irrespective of their absolute CD4 counts, in treatment naive patients with absolute CD4 cell counts more than 500 cells/mm few physician may choose to start antiretroviral therapy later until HCV treatment gets completed (CIII).

In patients with lower CD4 cell counts like <200 cells/ cubic mm,the antiretroviral therapy should be initiated promptly (AI) and treatment for HCV must be delayed until the patient is stable with HIV treatment (CIII).

MYCOBACTERIUM TUBERCULOSIS DISEASE WITH HIV COINFECTION

Principles for treatment of active tuberculosis disease in HIV infected individuals are the same as those for HIV uninfected individuals (AI).

In all HIV infected individuals with active TB infection should be started on TB treatment immediately following diagnosis (AI). All HIV infected individuals with active TB infection should be treated with antiretroviral therapy (ART) (AI).

In individuals with CD4 counts less than 50 cells/ cubic mm, ART should be started within 2 weeks of starting anti tuberculosis treatment (AI).

In individuals with absolute CD4 counts more than 50 cells/ cubicmmwho present with severe clinical disease like low Karnofsky score, low BMI, low albumin,low hemoglobin, organ system dysfunction, or the extent of the disease.Antiretroviral therapy should be started within 2 to 4 weeks of starting ATT. Strength of this recommendation varies according to the absolute CD4 cell counts:

- for absolute CD4 count 50 to 200 cells/ cubic mm (BI)
- for absolute CD4 count >200 cells/ cubic mm (BIII)

In patients with CD4 counts ≥50 cells/ cubic mmwho do not have severe clinical disease, ART can be delayed beyond 2 to 4 weeks of starting TB therapy but should be started within 8 to 12 weeks of ATT initiation. The strength of this recommendation also varies on the basis of absolute CD4 cell counts

- CD4 count 50 to 500 cells/ cubic mm (AI)
- CD4 count >500 cells/cubic mm (BIII)

In all HIV-infected pregnant women with active TB, ART should be started as early as possible, both for maternal health and for prevention of mother-to-child transmission of HIV (AIII). In HIV patients with documented multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, antiretroviral therapy should be started within 2 to 4 weeks of confirmation of TB drug resistance and initiation of second-line tuberculosis therapy (BIII).

Even there are pharmacokinetic drug interactions, rifamycin that is rifampin or rifabutin should be added in TB regimens for patients who is on ART,with dosage adjustment if needed (AII).

In HIV-infected patients with active tuberculosis disease who is on a protease inhibitor based regimen rifabutin is preferred over rifampin due to drug interactions (AII). But co-administration of rifampin and PIs with or without ritonavir boosting is not recommended by the panel (AII).IRIS, immune reconstitution inflammatory syndrome can occur after starting ART. But both ART and TB treatment has to be continued while managing IRIS (AIII).

RESULTS

This study mainly aimed at viewing the haematological abnormalities ongoing in HIV infection and the impaction of these abnormalities in viral markers like absolute CD4 count and HIV viral load. We were successfully able to complete the study among 100 HIV infected individuals attending in our hospital. It was a cross sectional study done among both outpatient and in inpatients with their consent. The protocol mentioned earlier was followed in all the patients. Statistical analysis of the study were given below:

TABLE 1:

HAEMOGLOBIN COUNT VS ABSOLUTE CD4 COUNT

	ABSOLUTE CD4 COUNT			
HAEMOGLOBIN COUNT	**>500 Cells / µl**	**200-500 Cells / µl**	**<200 Cells / µl**	**P value**
>12 GM/DL	21 (39.6%)	14 (26.4%)	18 (34.0%)	0.004
10-12 GM/DL	5 (22.7%)	4 (18.2%)	13 (59.1%)	
8-10 GM/DL	0 (0%)	3 (16.7%)	15 (83.3%)	
<8 GM/DL	0 (0%)	2 (28.6%)	5 (71.4%)	

FIGURE 1:

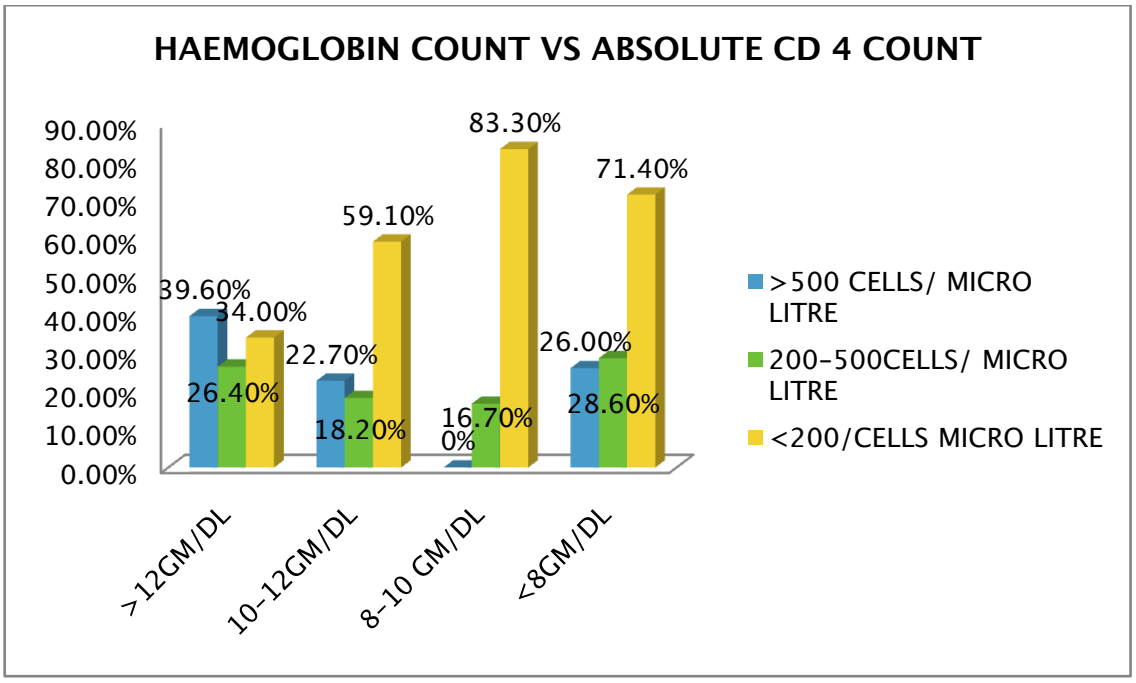
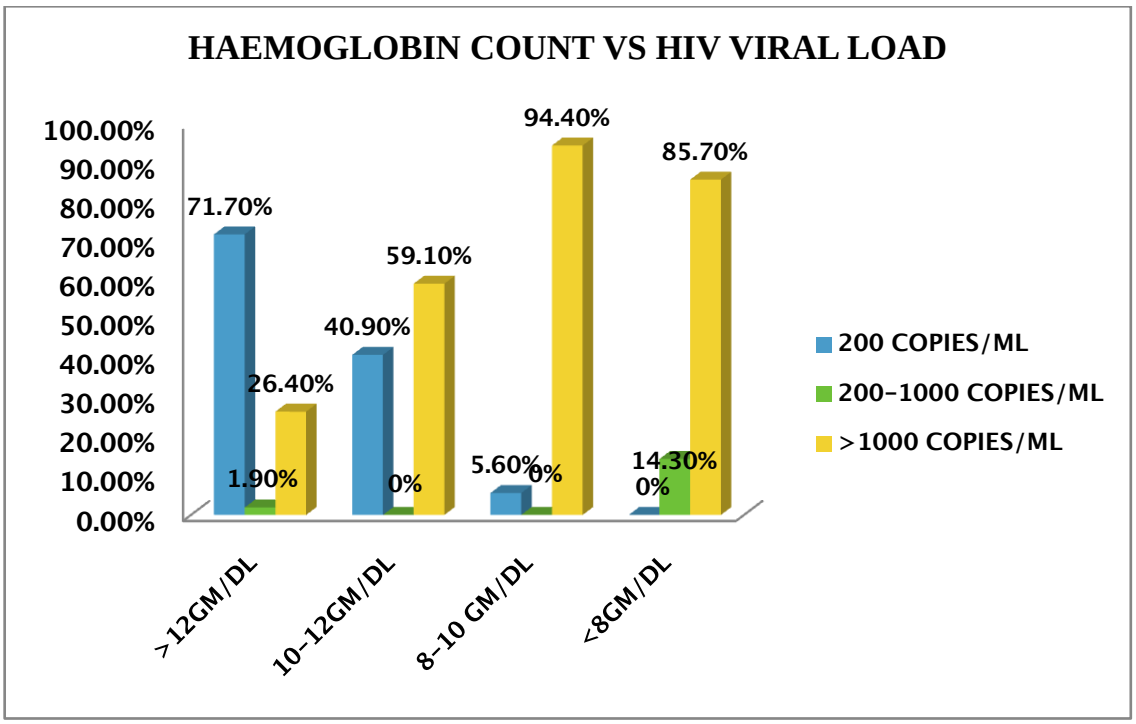


TABLE 2:

HAEMOGLOBIN COUNT VS HIV VIRAL LOAD

	HIV VIRAL LOAD			
HAEMOGLOBIN COUNT	**<200 copies/ml**	**200-1000 copies/ml**	**>1000 copies/ml**	**P value**
>12 GM/DL	38 (71.7%)	1 (1.9%)	14 (26.4%)	0.000
10-12 GM/DL	9 (40.9%)	0 (0%)	13 (59.1%)	
8-10 GM/DL	1(5.6%)	0 (0%)	17 (94.4%)	
<8 GM/DL	0 (0%)	1 (14.3%)	6 (85.7%)	

FIGURE 2:



ANAEMIA

In this study there were significant association noted between low haemoglobin count and absolute CD4 count and HIV viral load. Individuals with high viral load and low CD4 count < 200 cells/ microlitre had higher prevalence of anaemia. Severe anaemia was noted about 71.4% among CD 4 count < 200 cells/ microlitre, 28.6% among CD 4 count 200-500 cells/ microlitre and about 26% among CD 4 count >500 cells/ microlitre, as shown in Table 1.Severe anaemia was noted about 85.7% among HIV viral load >1000 copies/ ml, as shown in Table 2. Statistical analysis showed signicant P value among haemoglobin count and absolute CD4 count of 0.004 and with HIV viral load, P value was about 0.000.

TABLE 3:

HAEMOGLOBIN COUNT VS ABSOLUTE CD4 COUNT

(in treatment naive patients)

	ABSOLUTE CD4 COUNT			
HAEMOGLOBIN COUNT (naive patients)	**>500 cells/ µl**	**200-500 cells/ µl**	**<200 cells/ µl**	**P value**
>12 GM/DL	17 (39.5%)	13 (30.2%)	13 (30.2%)	0.008
10-12 GM/DL	3 (15.8%)	3 (15.8%)	13 (68.4%)	
8-10 GM/DL	0 (0%)	3 (23.1%)	10 (76.9%)	
<8 GM/DL	0 (0%)	2 (33.3%)	4 (66.7%)	

FIGURE 3:

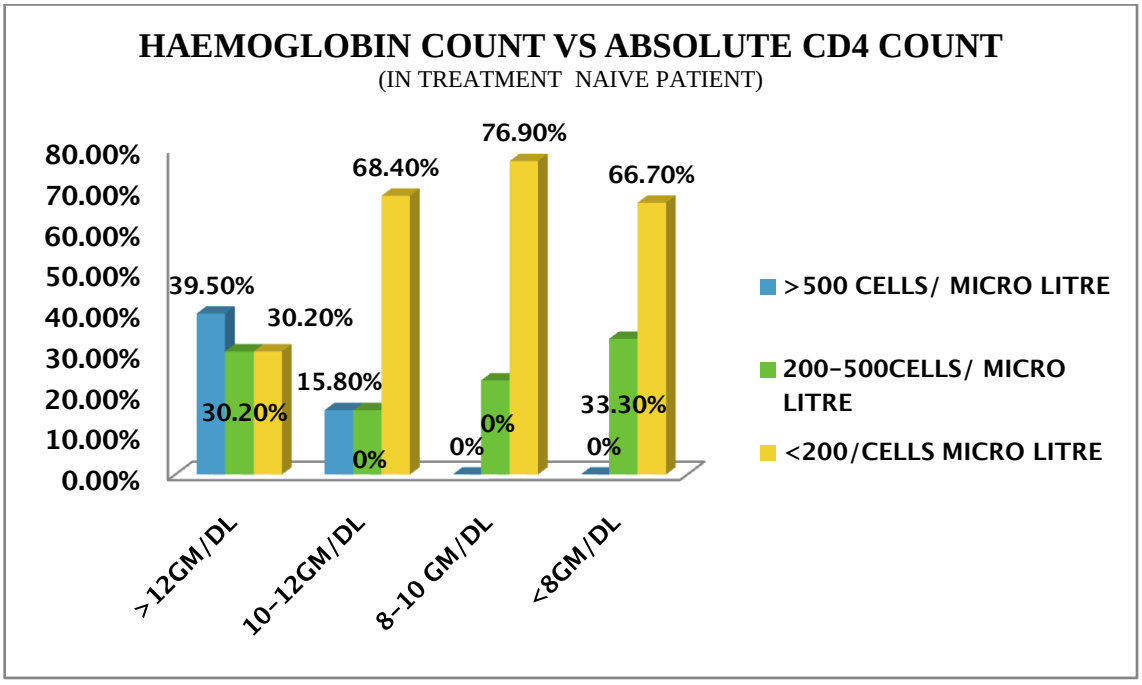


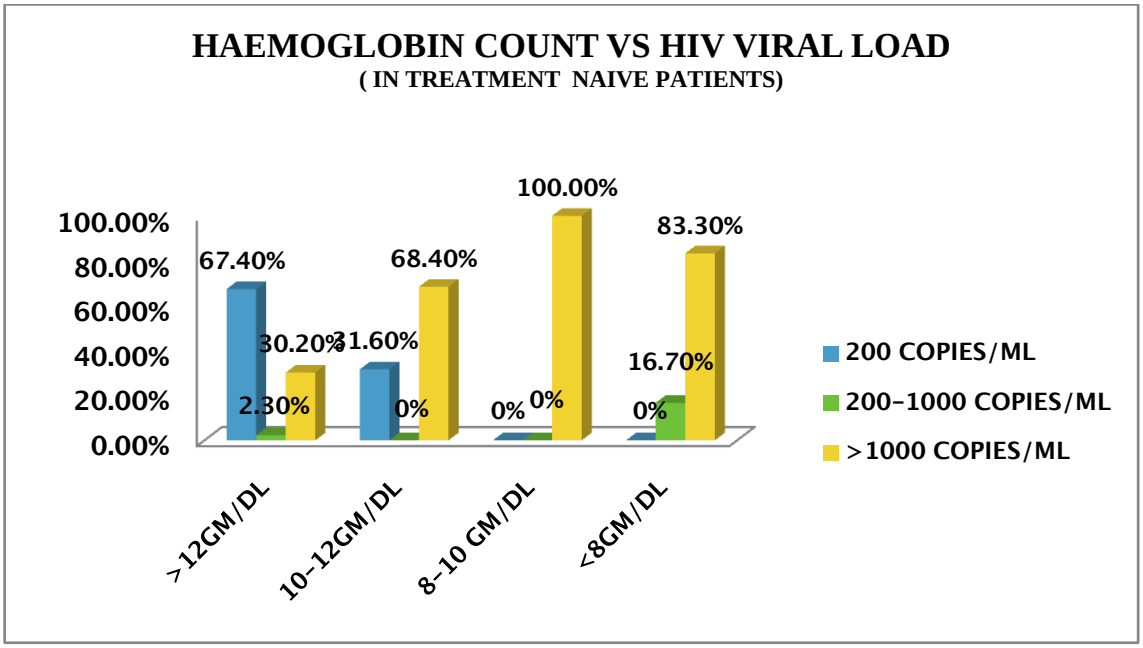
TABLE 4:

HAEMOGLOBIN COUNT VS HIV VIRAL LOAD

(in treatment naive patients)

	HIV VIRAL LOAD			
HAEMOGLOBIN COUNT (naive patients)	**<200 copies/ml**	**200-1000 copies/ml**	**>1000 copies/ml**	**P value**
>12 GM/DL	29 (67.4%)	1 (2.3%)	13 (30.2%)	0.000
10-12 GM/DL	6 (31.6%)	0 (0%)	13 (68.4%)	
8-10 GM/DL	0 (0%)	0 (0%)	13 (100.0%)	
<8 GM/DL	0 (0%)	1 (16.7%)	5 (83.3%)	

FIGURE 4:



In this study there were 81 treatment naïve individuals. So that statistical analysis were done separately among naïve patients. Treatment naïve individuals with high viral load and low CD4 count < 200 cells/ micro litre had higher prevalence of anaemia. Severe anaemia was noted about 66.7% among CD 4 count < 200 cells/ microlitre, 33.3% among CD 4 count 200-500 cells/ microlitre and about 0% among CD 4 count >500 cells/ microlitre,as shown in Table 3. Severe anaemia was noted about 83.3% among HIV viral load >1000 copies/ ml and 0 % among HIV viral load <200 copies/ ml, as shown in Table 4.Statistical analysis showed signicant p value among haemoglobin count in treatment naïve patients and absolute CD4 count of about 0.008 and with HIV viral load P value was about 0.000.

TABLE 5:

PLATELET COUNT VS ABSOLUTE CD4 COUNT

	ABSOLUTE CD4 COUNT			
PLATELET COUNT	**>500 cells/ µl**	**200-500 cells /µl**	**<200 cells/ µl**	**P value**
50000-100000 /µl	26 (27.7%)	22 (23.4%)	46 (48.9%)	0.511
20000-50000/ µl	0 (0%)	0 (0%)	1 (100.0%)	
<20000/ µl	0 (0%)	1 (20%)	4 (80.0%)	

FIGURE 5:

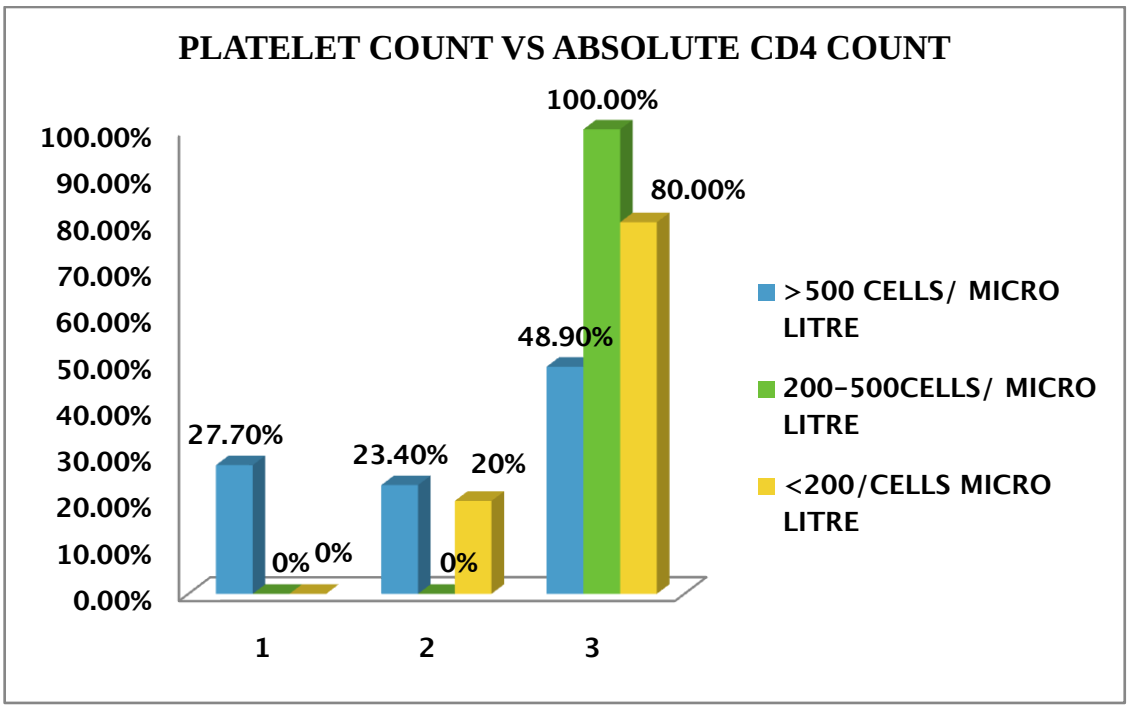


TABLE 6:

PLATELET COUNT VS ABSOLUTE CD4 COUNT

(in treatment naive patients)

	ABSOLUTE CD4 COUNT			
PLATELET COUNT (naive patients)	**>500 cells/ µl**	**200-500 cells /µl**	**<200 cells/ µl**	**P value**
50000-100000 /µl	20 (25.0%)	21 (26.3%)	39 (48.8%)	0.595
20000-50000/ µl	0 (0%)	0 (0%)	0 (0%)	
<20000/ µl	0 (0%)	0 (0%)	1 (100.0%)	

FIGURE 6:

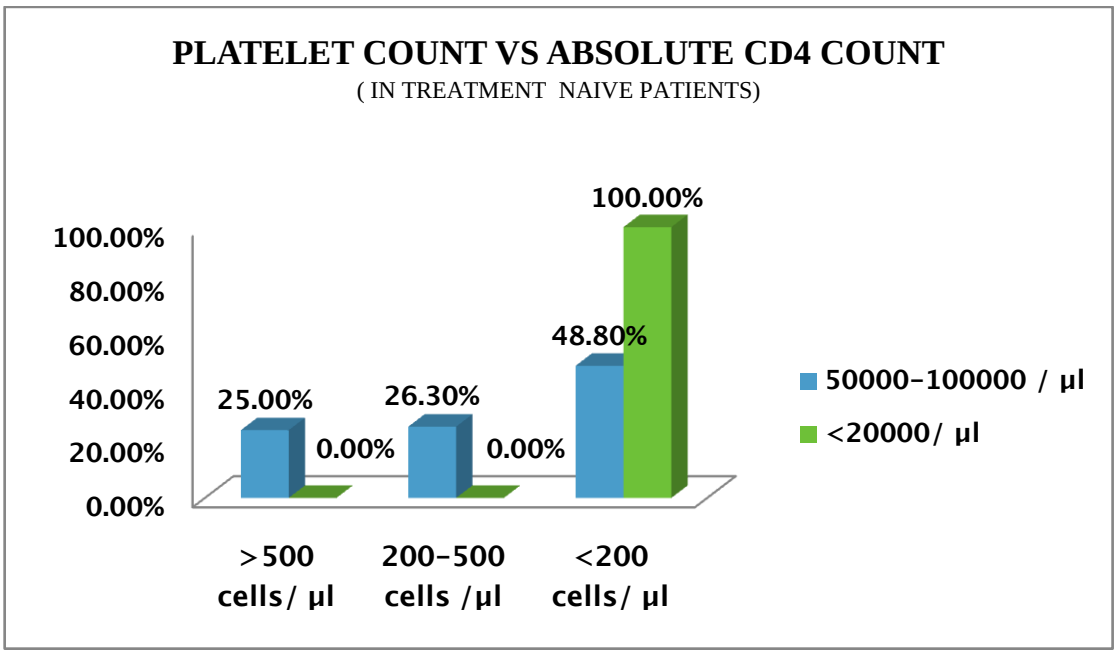


TABLE 7:

PLATELET VS HIV VIRAL LOAD

	HIV VIRAL LOAD			
PLATELET COUNT	**<200 copies/ml**	**200-1000 copies/ml**	**>1000 copies/ml**	**P value**
50000-100000 / µl	47 (50.0%)	2 (2.1%)	45 (47.9%)	0.560
20000-50000/ µl	0 (0%)	0 (0%)	1 (100.0%)	
<20000/ µl	1 (20.0%)	0 (0%)	4 (80.0%)	

FIGURE 7:

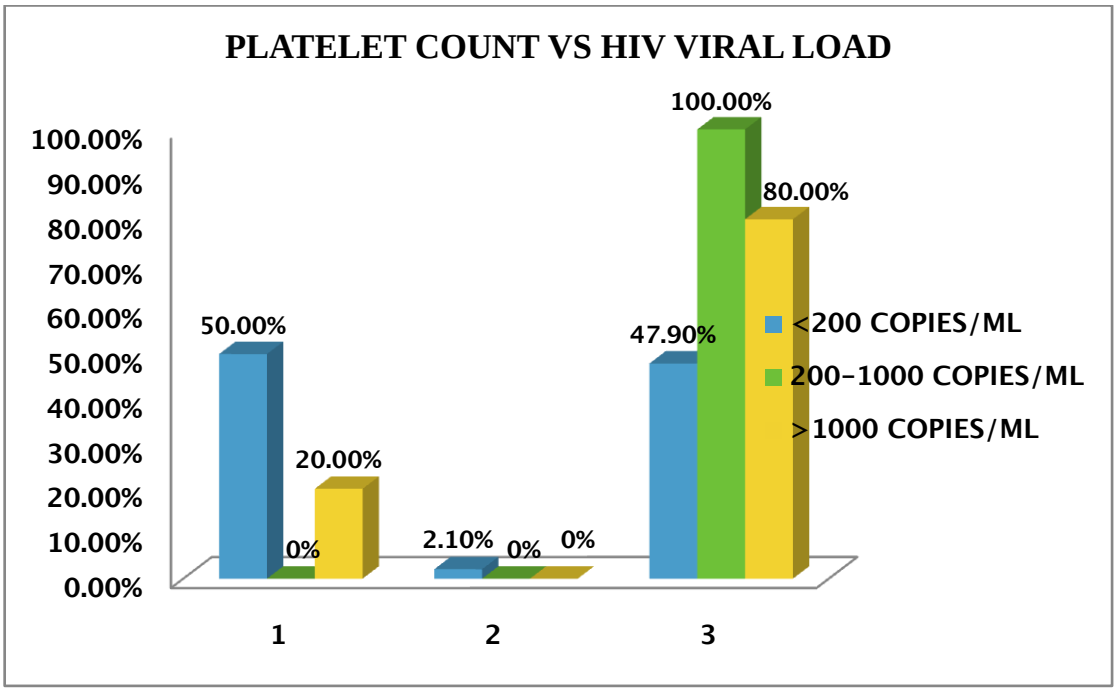


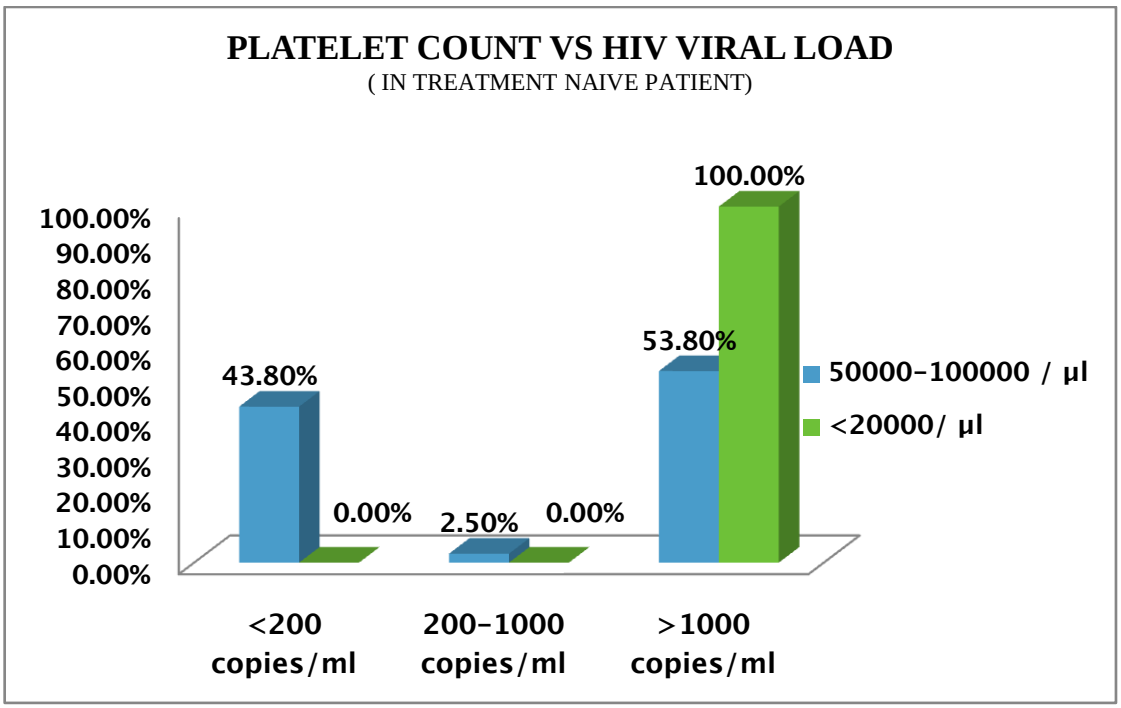
TABLE 8:

PLATELET COUNT VS HIV VIRAL LOAD

(in treatment naive patient)

	HIV VIRAL LOAD			
PLATELET COUNT (naive patient)	**<200 copies/ml**	**200-1000 copies/ml**	**>1000 copies/ml**	**P value**
50000-100000 / µl	35 (43.8%)	2 (2.5%)	43 (53.8%)	0.653
20000-50000/ µl	0 (0%)	0 (0%)	0 (0%)	
<20000/ µl	0 (0%)	0 (0%)	1 (100.0%)	

FIGURE 8:



Among total samples, analysis of platelet count and absolute CD4 count shows a p value of 0.511 (Table 5) and in treatment naïve patients was 0.595 (Table 6), which were insignificantly associated .

Comparison of platelet count and HIV viral load shows a p value of 0.560 (Table 7) and in treatment naïve patients was 0.653 (Table 8), which were insignificant .

TABLE 9:

ABSOLUTE NEUTROPHIL COUNT VS ABSOLUTE CD4 COUNT

	ABSOLUTE CD4 COUNT			
ABSOLUTE NEUTROPHIL COUNT	**>500 cells/ µl**	**200-500 cells/ µl**	**<200 cells/ µl**	**P VALUE**
1000-1500 / µl	26 (28.3%)	22 (23.9%)	44 (47.8%)	0.266
500-1000/ µl	0 (0%)	1 (16.7%)	5 (83.3%)	
<500/ µl	0 (0%)	0 (0%)	2 (100.0%)	

FIGURE 9:

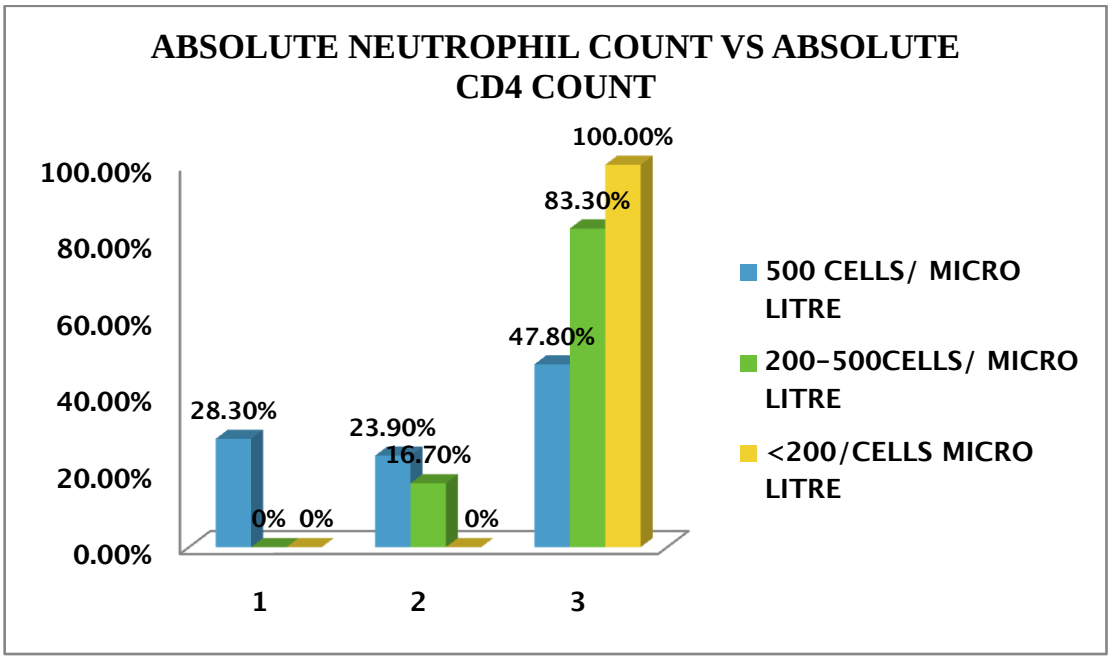


TABLE 10:

ABSOLUTE NEUTROPHIL COUNT VS ABSOLUTE CD4 COUNT

(In treatment naive patient)

	ABSOLUTE CD4 COUNT			
ABSOLUTE NEUTROPHIL COUNT (naïve patient)	**>500 cells/ µl**	**200-500 cells/ µl**	**<200 cells/ µl**	**P Value**
1000-1500 / µl	20 (27.0%)	20 (27.0%)	34 (45.9%)	0.345
500-1000/ µl	0 (0%)	1 (16.7%)	5 (83.3%)	
<500/ µl	0 (0%)	0 (0%)	1 (100.0%)	

FIGURE 10:

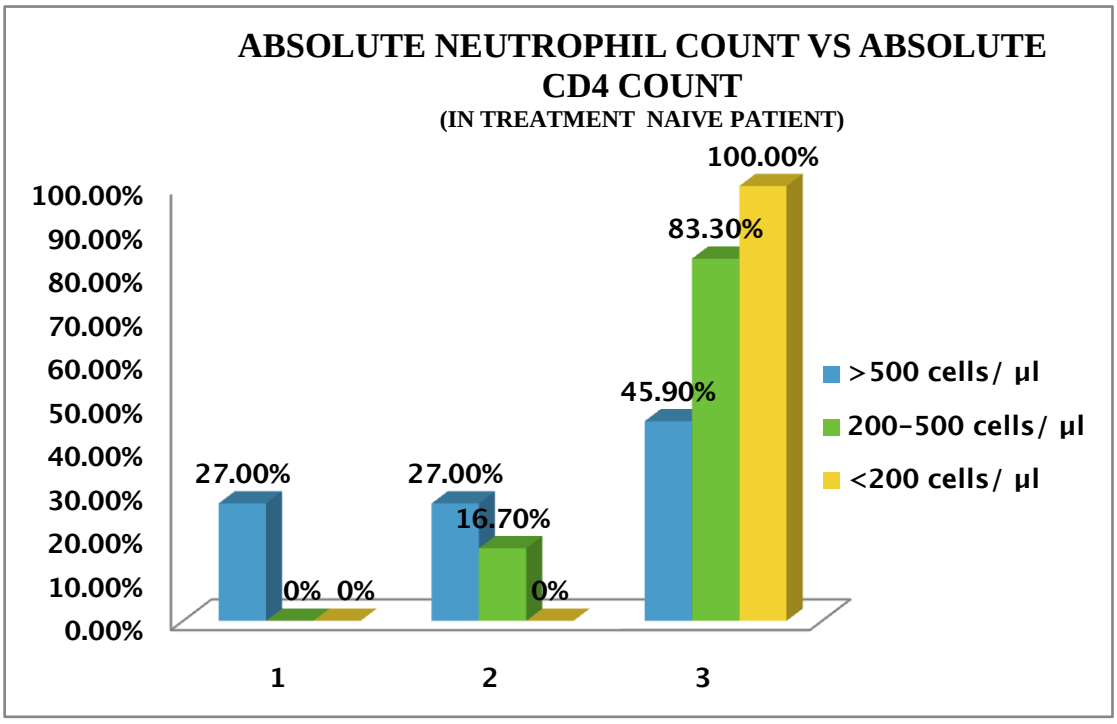


TABLE 11:

ABSOLUTE NEUTROPHIL COUNT VS HIV VIRAL LOAD

	HIV VIRAL LOAD			
ABSOLUTE NEUTROPHIL COUNT	**<200 copies/ml**	**200-1000 copies/ml**	**>1000 copies/ml**	**P value**
1000-1500 / µl	48 (52.2%)	2 (2.2%)	42 (45.7%)	0.069
500-1000/ µl	0 (0%)	0 (0%)	6 (100.0%)	
<500/ µl	0 (0%)	0 (0%)	2 (100.0%)	

FIGURE 11:

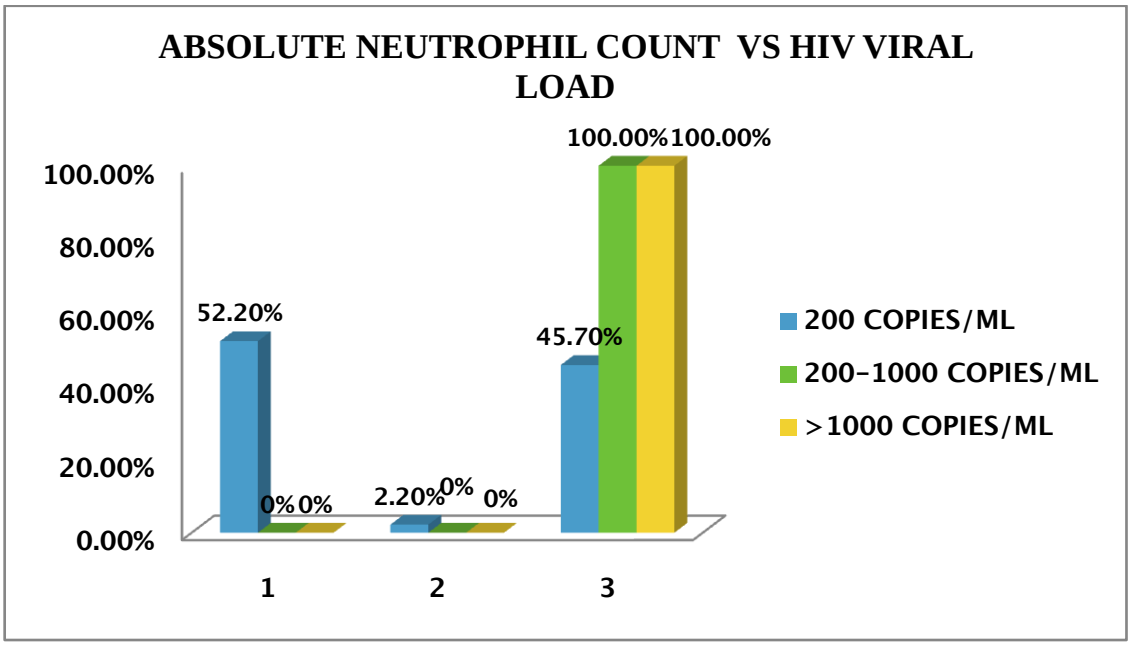


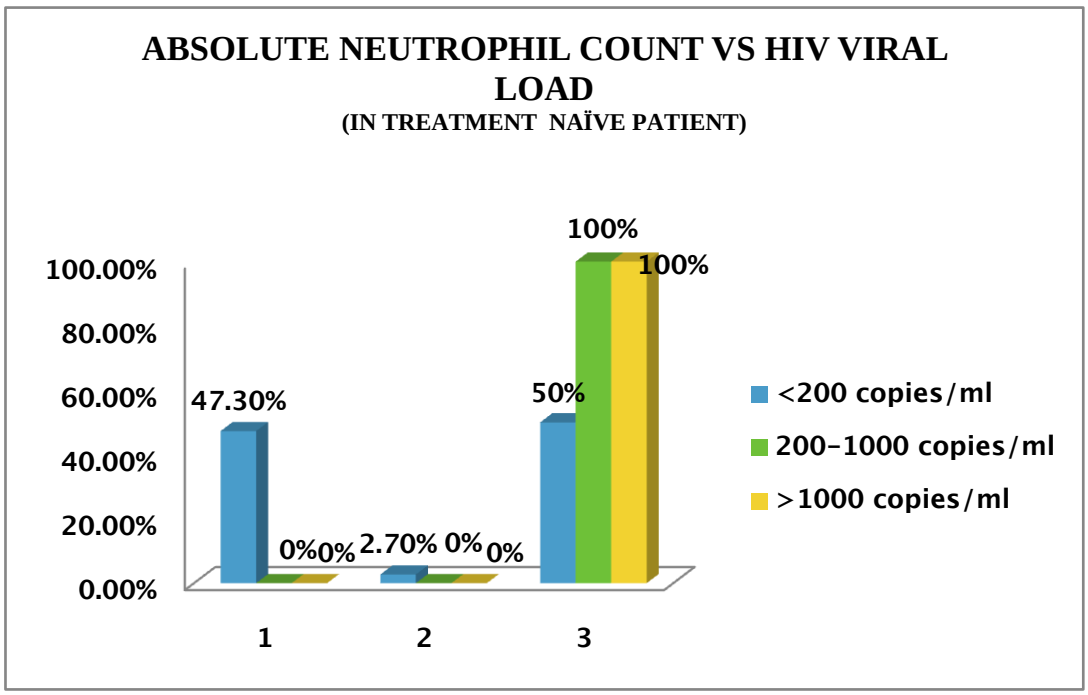
TABLE 12 :

ABSOLUTE NEUTROPHIL COUNT VS HIV VIRAL LOAD

(in treatment naïve patient)

	HIV VIRAL LOAD			
ABSOLUTE NEUTROPHIL COUNT	**<200 copies/ml**	**200-1000 copies/ml**	**>1000 copies/ml**	**P value**
1000-1500 / µl	35(47.3%)	2 (2.7%)	37 (50.0%)	0.168
500-1000/ µl	0 (0%)	0 (0%)	6 (100.0%)	
<500/ µl	0 (0%)	0 (0%)	1 (100.0%)	

FIGURE 12:



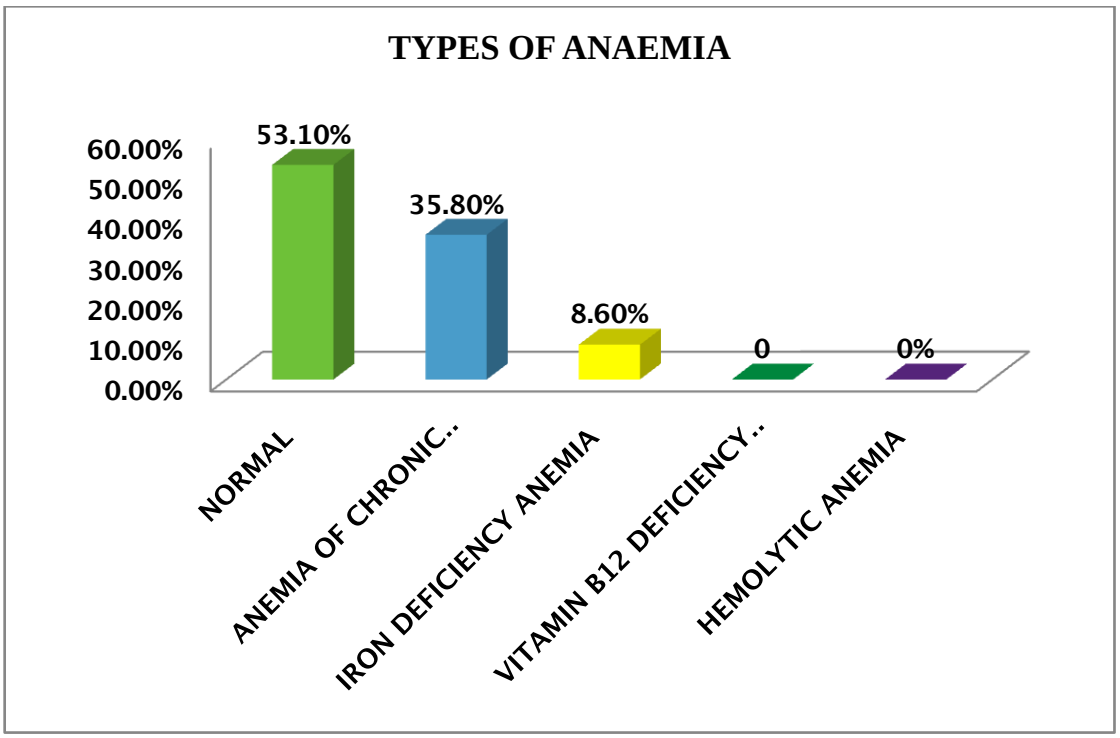
Among total samples, analysis of absolute neutrophil count and absolute CD4 count shows a p value of 0.266 (Table 9) and in treatment naïve patients was 0.345 (Table 10), which were insignificantly associated.

Comparison of absolute neutrophil count and HIV viral load shows a p value of 0.069 (Table 11) and in treatment naïve patients was 0.168 (Table 12), which were insignificant.

TABLE 13:

TYPE OF ANAEMIA	AMONG NAIVE PATIENTS
NORMAL	43 (53.1%)
ANAEMIA OF CHRONIC DISEASE	29 (35.8%)
IRON DEFICIENCY ANAEMIA	7 (8.6%)
VITAMIN B12 DEFICIENCY ANAEMIA	2 (2.5%)
HEMOLYTIC ANAEMIA	0 (0%)

FIGURE 13:



TYPE OF ANAEMIA:

In this study, normocytic normochromic blood picture was noted in majority of the patients, as shown in Table 13. Among the type of anaemia, anaemia of chronic disease blood picture was more prevalent among these individuals. It was about 35.8 % among the total cases. Iron deficiency anaemia was noted about 8.6% of the cases. Vitamin B12 deficiency was documented in 2% of the case. Hemolytic anaemia was not noted in this study.

TABLE 14:

ABSOLUTE CD4 COUNT VS COINFECTION

ABSOLUTE CD4 COUNT	CO-INFECTION ABSENT	CO-INFECTION PRESENT	P VALUE
>500 cells/ µl	22 (84.6%)	4 (15.4%)	0.000
200-500 cells/µl	1 (47.8%)	12 (52.2%)	
<200 cells/ µl	8 (16.0%)	42 (84.0%)	

FIGURE 14:

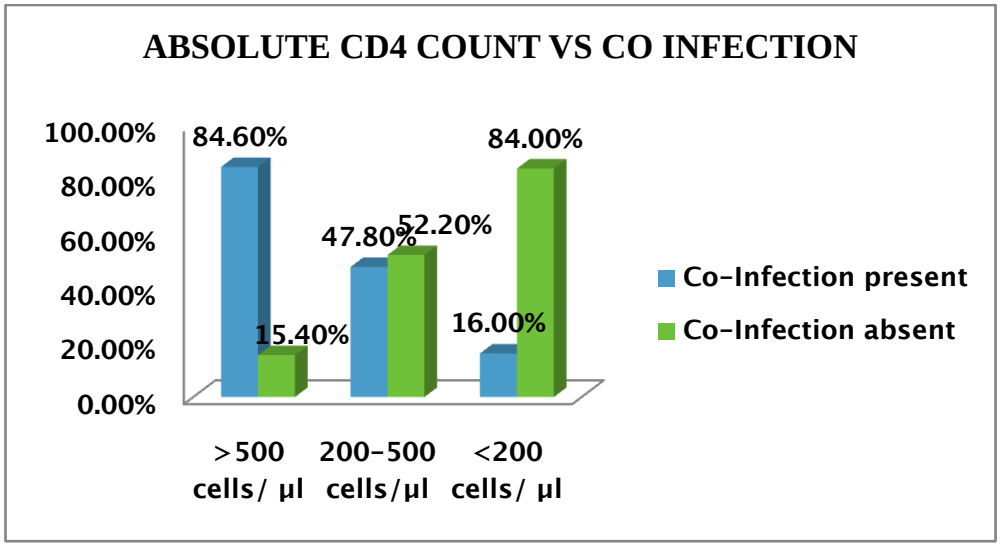


TABLE 15: HIV VIRAL LOAD VS CO INFECTION

HIV VIRAL LOAD	CO-INFECTION ABSENT	CO-INFECTION PRESENT	P Value
<200 copies/ml	34 (70.8%)	14 (29.2%)	0.000
200-1000 copies/ml	2 (100.0%)	0 (0%)	
>1000 copies/ml	5 (10.0%)	45 (90.0%)	

FIGURE 15:

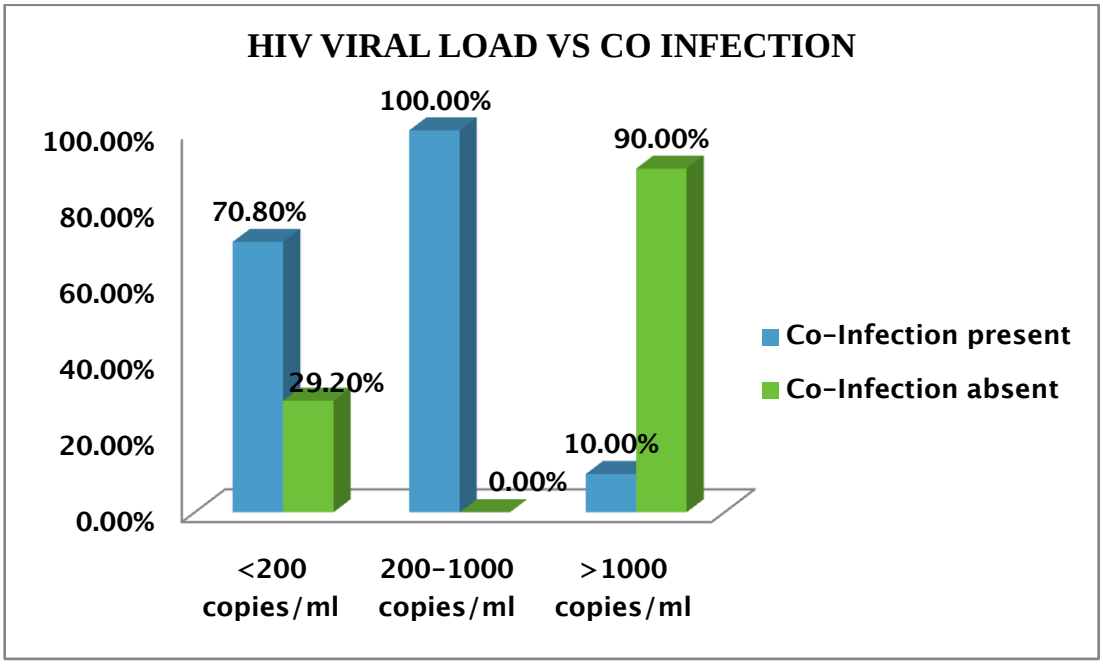
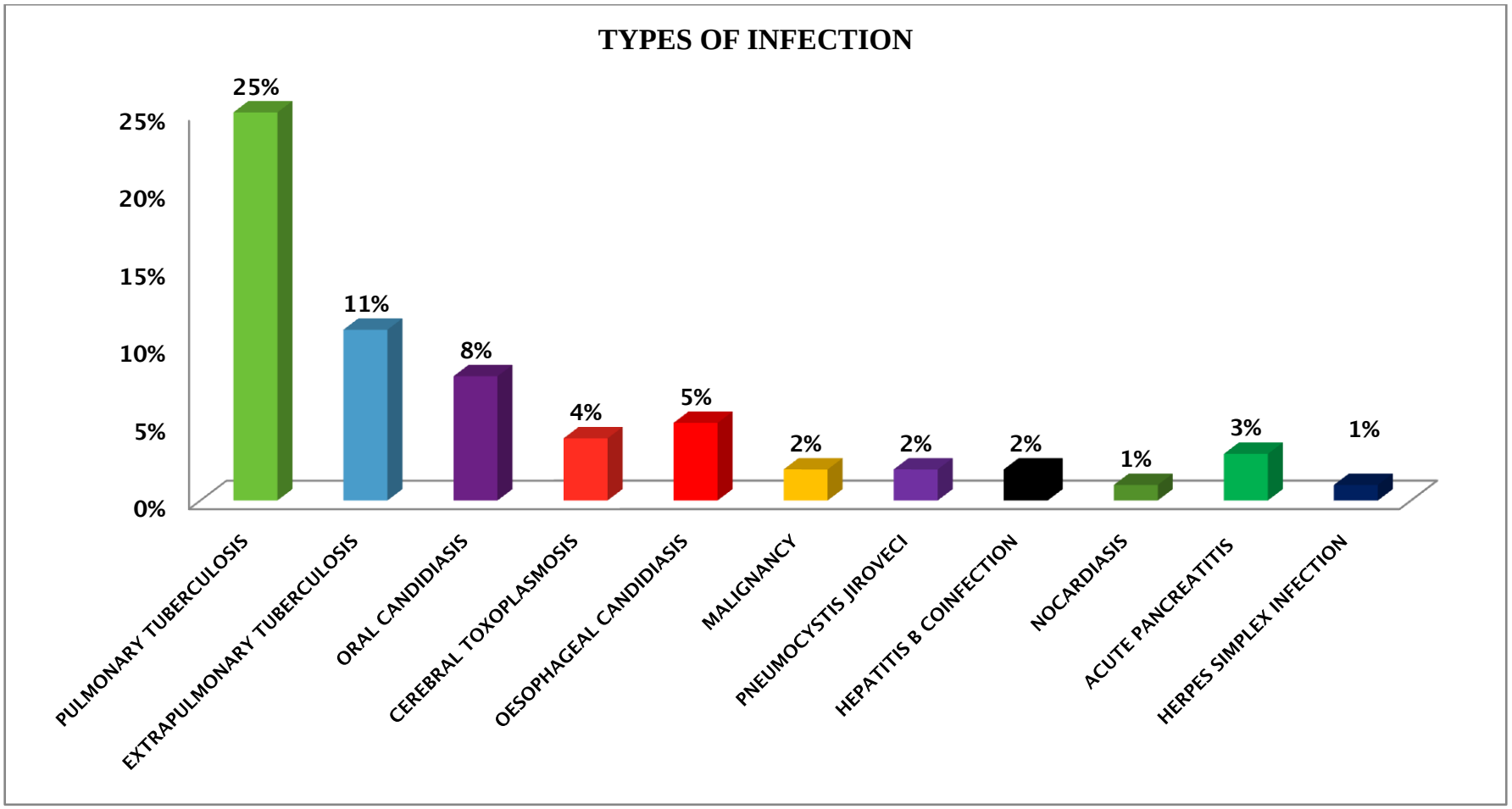


TABLE 16:

CO INFECTIONS

S.NO	TYPE OF INFECTION	FREQUENCY	PERCENTAGE
1	PULMONARY TUBERCULOSIS	25	25
2	EXTRAPULMONARY TUBER-CULOSIS	11	11
3	ORAL CANDIDIASIS	8	8
4	CEREBRAL TOXOPLASMOSIS	4	4
5	OESOPHAGEAL CANDIDIASIS	5	5
6	MALIGNANCY	2	2
7	PNEUMOCYSTIS JIROVECI	2	2
8	HEPATITIS B COINFECTION	2	2
9	NOCARDIASIS	1	1
10	ACUTE PANCREATITIS	3	3
11	HERPES SIMPLEX INFECTION	1	1

FIGURE 16:


TYPES OF INFECTION
25%
20%
15%
10%
5%
0%
25%
11%
8%
4%
5%
2%
2%
2%
1%
3%
1%
PULMONARY TUBERCULOSIS
EXTRAPULMONARY TUBERCULOSIS
ORAL CANDIDIASIS
CEREBRAL TOXOPLASMOSIS
OESOPHAGEAL CANDIDIASIS
MALIGNANCY
PNEUMOCYSTIS JIROVECI
HEPATITIS B COINFECTION
NOCARDIASIS
ACUTE PANCREATITIS
HERPES SIMPLEX INFECTION

COINFECTION

Comparison of occurrence of co infection and absolute CD4 count showed a p value of 0.000 (Table 14) and comparison of occurrence of co infection and HIV viral load showed a p value of 0.000 (Table 15). There were significant association noted between the occurrence of co infection and absolute CD4 count and HIV viral load. In individuals with high viral load and low CD4 count < 200 cells/ micro litre had higher rate of occurrence of co infection.

Among the co infections pulmonary tuberculosis were predominant and it was about 25% of the total. Extrapulmonary tuberculosis were noted about 11%. CNS toxoplasmosis were noted among 4 individuals. Oral candidiasis were about 8% and oesophageal candidiasis were about 5%. Other co infections noted were pneumocystis jiroveci infection, nocardiasis, herpes simplex infection.All were about less than 5%. Malignancies and hepatitis B co infection were noted about 2 % of the total population. Acute pancreatitis were noted about 3 % of the cases.

BONE MARROW BIOPSY:

In this study, bone marrow biopsy was done among 5 patients. Among 3 individuals , the bone marrow picture was erythroid hyperplasia with mild dyserythropoiesis, megakaryocytic hyperplasia and mild increase in plasma cells which were suggestive of HIV infection. In 2 of the individuals, immune thrombocytopenia were considered.

Nearly 10 patients had pancytopenia picture. In this study about 81 patients were treatment naïve and 19 were on anti retroviral therapy. Among the individuals on anti retroviral therapy,those patients who were on zidovudine based therapy were having normocytic normochromic anaemia with macrocytosis.

DISCUSSION

This study among HIV infected individuals from South India showed prevalence of anaemia about 47% as compared to Mildvan et alwho found the prevalence of anemia in 9690 HIV infected patients as 39.5%. In this study, anaemia was more prevalent in individuals with high HIV viral load and low absolute CD4 count .This was the same like many studies quoted in the literature review. Even the severity of anaemia was significantly correlated with high HIV viral load and low absolute CD4 count. These findings are consistent with study done by Volberding et al., who reported that more severe levels of anemia are found among HIV positive patients presenting with low CD4 counts.

Among the total cases, anaemia was more common than thrombocytopenia or neutropenia.In treatment naïve individuals and individuals on ART, thrombocytopenia and neutropenia were more prevalent among high HIV viral load and low CD4 count < 200 cells/ microlitre. But statistical analysis showed insignicant P value in both the groups.

The incidence and severity of anaemia, thrombocytopenia and neutropenia were reflecting the underlying immune status if interpreted cautiously. It will be of great benefit especially if the patient is in regular follow-up. Hence it is necessary to identify and treat for hematological abnormalities to reduce the morbidity and mortality.

CONCLUSION

Though there were many studies been conducted across India on HIV manifestations, in majority of them, various aspects were addressed and the prime focus on the hematological manifestations were very limited. Most of the available data what we presently have is from the western studies, which might not be directly applicable to our Indian population. Hence this study was planned and done in Indian population mainly from South India. This study looked into the hematological manifestations and their correlation with viral markers.

HIV viral load and absolute CD 4 count are the most essential biomarkers correlating to the stage of HIV disease and its progression. But these tests are costlier. Even developed nations were finding it difficult. So country like ours definitely requires other alternatives to reduce the economical burden by frequent testing. For the sake of economical evaluation of the status of HIV disease stage and its progression, complete blood counts and peripheral smear has been suggested as the alternatives, since they were significantly correlated with high HIV viral load and lower absolute CD4 cell counts.

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INFORMED CONSENT

PSG Institute of Medical Science and Research, Coimbatore

Institutional Human Ethics Committee

INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

I / We (write name of the investigator(s) here), Dr. S.Suja, am carrying out a study on the topic: :

Profile of hematological abnormalities and its correlation with absolute CD4 count and HIV viral load in HIV infected patients in a tertiary care hospital

as part of my / our research project being carried out under the aegis of the Department of: General medicine

(*Applicable to students only*): My / our research guide is: Dr. T.Saravanan MD.

The justification for this study is:

Though many studies have been conducted on various manifestations of HIV disease, the focus on hematological abnormalities in these studies is very little. Also there are very few literature in Indian population correlating hematological abnormalities to absolute CD4 count , HIV viral load and opportunistic infections. Most of the available data is from the west, which might not be directly applicable to our Indian population.Therefore this study would evaluate the correlation in HIV to absolute CD4 count and viral load.

Ojectives of the study are:

Primary objective:

To study the spectrum of hematological abnormalities in HIV infected patients

Secondary objective:

To find the correlation of hematological abnormalities with absolute CD4 count and HIV viral load

Sample size: 100

Study volunteers / participants are (specify population group & age group): patients above age of 16 years who have been diagnosed of HIV infection either by ELISA or western blot method.

Location: PSG hospitals , Coimbatore.

Data collected will be stored for a period of three years. We will / will not use the data as part of another study.

Blood sample collection: Specify quantity of blood being drawn: __10__ml.

No. of times it will be collected: Once or twice over 2 days.

Whether blood sample collection is part of routine procedure or for research (study) purpose: Routine procedure

1. Routine procedure 2. Research purpose

Specify **purpose**, discomfort likely to be felt and side effects, if any: __Nil___

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: No

Whether blood sample collected will be shared with persons from another institution: No

Medication given, if any, duration, side effects, purpose, benefits: Nil

Benefits from this study: .Effective treatment to bring down the morbidity and mortality

Risks involved by participating in this study: Nil

How the **results** will be used: Study will be submitted to Dr. MGR Medical University as thesis in post graduate course in general medicine.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time

you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings- including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI: 9965584827

Contact number of Ethics Committee Office: 0422 2570170 Extn: 5818

ஒப்புதல் படிவம்

தேதி:

ச.சுஜா ..... ஆகிய நான் PSG மருத்துவக் கல்லூரியின் பொது மருத்துவத் துறையின் கீழ் எச்.ஐ.வி கிருமியால் பாதிக்கப்பட்டுள்ள நோயாளிகளில் ஏற்படும் இரத்த சம்பந்தமான கோளாறுகளும் மற்றும் இவைகளுக்கும் CD4 அணுக்கள் மற்றும் எச்.ஐ.வி வைரஸ் எண்ணிக்கை ஆகியவற்றுக்குள் இருக்கும் தொடர்பை கண்டறிவது என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்.

என் ஆய்வு வழிகாட்டி: த.சரவணன்

ஆய்வு மேற்கொள்வதற்கான அடிப்படை: எச்.ஐ.வி கிருமியால் ஏற்படும் இரத்த சம்பந்தமான கோளாறுகளைப் பற்றியும் மேலும் இந்திய மக்களில் இம்மாதிரியான ஆய்வுகளும் மிகக் குறைவாகவே உள்ளன.ஆகவே இந்த ஆய்வை மேற்கொள்ள விரும்புகிறோம்.

ஆய்வின் நோக்கம் :

1. எச்.ஐ.வி கிருமியால் பாதிக்கப்பட்டுள்ள நோயாளிகளில் ஏற்படும் இரத்த சம்பந்தமான கோளாறுகளை கண்டறிவது.
2. இரத்த சம்பந்தமான கோளாறுகளுக்கும் , CD4 அணுக்கள் மற்றும் எச்.ஐ.வி கிருமிகளின் எண்ணிக்கை ஆகியவற்றுக்குள் இருக்கும் தொடர்பை கண்டறிவது.

ஆய்வின் பங்கு பெறும் நபர்களின் எண்ணிக்கை:100

ஆய்வு மேற்கொள்ளும் இடம்: பூ.சா.கோ மருத்துவமனை

ஆய்வின் பலன்கள்: உரிய மருத்துவ சிகிச்சையின் மூலம் நோயினால் ஏற்படும் பாதிப்புகளை குறைக்க முடியும்.

ஆய்வினால் ஏற்படும் அசௌகரியங்கள்/ பக்க விளைவுகள்: ஏதுமில்லை

இந்த ஆய்வில் கிடக்கும் தகவல்கள் 3 வருடங்கள் பாதுகாக்கப்படும்.இவை வேறு எந்த ஆய்விற்கும் பயன்படுத்தப் பட மாட்டாது.எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்பட மாட்டாது.அவை இரகசியமாக வைக்கப்படும்.

MASTER CHART

Sl. No.	ABSOLUTE NEUTROPHIL COUNT	HAEMOGLOBIN COUNT	PLATELET COUNT	PERIPHERAL SMEAR	TYPE OF ANAEMIA	TREATMENT HISTORY	ABSOLUTE CD4 COUNT	HIV VIRAL LOAD	WBC	CO INFECTION	BONE MARROW
1	1	1	1	1	0	0	2	1	1	7	-
2	1	1	3	3	0	1	3	3	1	9	-
3	1	1	1	3	0	1	3	1	1	1	-
4	1	2	1	2	2	0	3	3	1	1 ,5	-
5	2	2	1	3	1	0	3	3	2	10	-
6	1	1	1	1	0	0	1	1	0	0	-
7	1	3	1	1	2	0	3	3	1	1	-
8	1	1	1	1	0	0	2	3	0	1	-
9	1	3	1	1	1	0	2	3	1	1	-
10	1	3	1	1	1	0	2	3	1	1	-
11	1	2	1	1	1	0	3	3	0	0	-
12	1	2	1	1	1	0	2	3	1	1	-
13	1	2	1	1	1	0	1	3	1	1	-
14	1	1	1	1	0	0	2	3	1	1	-
15	2	4	1	1	1	0	2	3	2	4,2	-
16	1	2	3	1	1	1	2	1	1	0	-
17	1	1	1	1	0	0	3	1	1	13	2
18	1	1	1	1	0	0	3	1	1	8	-
19	1	1	1	1	0	1	2	1	1	0	-
20	1	2	1	1	1	0	3	3	1	1	-
21	2	4	1	2	1	0	3	3	2	1	-
22	2	3	1	1	2	0	3	3	2	2	-
23	1	1	1	1	0	0	3	3	1	1,3	-
24	1	2	1	1	1	0	3	3	1	1	-
25	2	3	1	2	2	0	3	3	2	1	-
26	1	4	1	1	1	0	2	2	1	0	-
27	1	2	1	1	1	0	2	1	0	2	-
28	1	2	1	1	1	0	3	3	0	1	-
29	1	2	1	1	1	0	3	3	0	1	-
30	1	1	1	1	0	0	3	1	1	0	-
31	1	1	1	1	0	0	3	1	1	0	-
32	1	1	1	3	0	0	3	1	1	1	-
33	1	1	1	1	0	1	1	1	1	2	-
34	1	1	1	1	0	1	1	1	1	0	-
35	1	1	1	1	0	1	3	1	1	11	-
36	1	2	1	1	1	0	3	1	2	1	-
37	1	4	1	1	2	0	3	3	2	2,8	-

Sl. No.	ABSOLUTE NEUTROPHIL COUNT	HAEMOGLOBIN COUNT	PLATELET COUNT	PERIPHERAL SMEAR	TYPE OF ANAEMIA	TREATMENT HISTORY	ABSOLUTE CD4 COUNT	HIV VIRAL LOAD	WBC	CO INFECTION	BONE MARROW
38	1	1	1	3	0	1	1	1	0	0	1
39	1	1	1	1	0	0	1	1	1	0	-
40	1	1	1	1	0	1	3	1	0	11	-
41	1	3	1	1	1	1	3	3	1	2	-
42	1	1	1	1	0	0	3	3	2	1,4	-
43	1	3	1	2	2	0	2	3	1	11	-
44	1	3	1	1	1	0	3	3	1	1	-
45	3	4	1	1	0	0	3	3	2	1,8	-
46	1	2	1	1	1	0	3	3	1	6	1
47	1	2	1	1	1	0	1	3	0	4	-
48	1	1	1	1	3	0	3	3	1	6	-
49	1	1	1	1	0	0	1	1	1	9	-
50	1	3	1	1	1	0	3	3	1	1,4	-
51	1	1	1	1	0	0	3	3	1	1	-
52	2	1	1	1	0	0	3	3	2	4	-
53	1	2	1	1	1	0	1	1	0	0	-
54	1	2	1	1	1	1	1	1	1	0	-
55	1	2	1	1	1	1	1	1	1	0	-
56	1	4	1	2	1	0	3	3	0	1	-
57	1	1	1	1	0	0	2	3	1	1	-
58	1	3	1	2	2	1	3	1	2	2	-
59	1	2	1	3	1	0	3	1	1	0	-
60	1	1	1	1	0	0	1	1	1	0	-
61	1	3	1	1	0	0	3	3	1	4	-
62	1	3	3	1	1	1	3	3	1	2	-
63	1	1	1	1	0	0	1	1	1	0	1
64	1	1	1	1	0	0	3	3	1	8,3	-
65	1	1	1	3	0	1	3	1	1	0	-
66	1	1	1	1	0	1	1	1	1	0	-
67	4	4	1	2	2	1	3	3	4	2	2
68	1	2	1	1	1	0	3	1	0	1	-
69	1	3	1	2	2	0	3	3	0	12,3	-
70	1	2	1	1	1	0	3	3	1	0	-
71	1	3	2	3	1	1	3	3	2	1	-
72	1	1	1	1	1	0	1	1	1	0	-
73	1	1	1	3	0	0	2	1	1	0	-
74	1	1	1	1	0	0	1	1	1	0	-
75	1	1	1	1	0	0	1	1	1	0	-
76	1	1	1	1	0	0	1	1	1	0	-

Sl. No.	ABSOLUTE NEUTROPHIL COUNT	HAEMOGLOBIN COUNT	PLATELET COUNT	PERIPHERAL SMEAR	TYPE OF ANAEMIA	TREATMENT HISTORY	ABSOLUTE CD4 COUNT	HIV VIRAL LOAD	WBC	CO INFECTION	BONE MARROW
77	1	1	1	1	0	0	1	2	1	0	-
78	1	**3**	**3**	**1**	**1**	**1**	**3**	**3**	**1**	**1**	-
79	1	1	1	1	0	0	2	1	0	1	-
80	1	1	1	1	0	0	1	1	1	0	-
81	1	1	1	1	0	0	1	1	1	0	-
82	1	1	1	3	0	0	2	1	1	0	-
83	1	1	1	1	0	0	1	1	1	0	-
84	1	1	1	1	0	0	1	1	1	0	-
85	1	1	1	1	0	0	1	1	1	0	-
86	1	2	1	1	1	0	3	3	2	2	-
87	1	1	1	1	0	0	2	1	1	0	-
88	1	1	1	1	0	0	2	1	1	0	-
89	1	3	1	1	1	0	3	3	0	3,5	-
90	1	1	1	1	0	0	2	3	0	0	-
91	1	1	1	1	0	0	3	3	0	0	-
92	1	1	3	1	0	0	3	3	1	0	-
93	1	1	1	1	0	0	2	1	1	0	-
94	1	1	1	1	0	0	1	1	1	0	-
95	1	3	1	1	1	0	3	3	0	1	-
96	1	2	1	1	3	0	2	1	1	0	-
97	1	1	1	1	0	0	2	3	1	7	-
98	1	1	1	1	0	0	1	1	1	0	-
99	1	1	1	1	0	0	2	1	1	0	-
100	1	3	1	1	1	0	3	3	0	2	-

MASTER CHART KEY

HAEMOGLOBIN COUNT

1 NORMAL >12 GM/DL
2 MILD 10-12 GM/DL
3 MODERATE 8-10 GM/DL
4 SEVERE <8 GM/DL

PLATELET COUNT

THROMBOCYTOPENIA
1 MILD 50000-100000/ µl
2 MODERATE 20000-50000/ µl
3 SEVERE <20000 / µl

ABSOLUTE NEUTROPHIL COUNT

1 MILD NEUTROPENIA 1000-1500/ µl
2 MODERATE 500-1000/ µl
3 SEVERE <500/ µl

TYPE OF ANAEMIA

1 NORMAL
2 ANAEMIA OF CHRONIC DISEASE
3 IRON DEFICIENCY ANAEMIA
4 VITAMIN B12 DEFICIENCY ANAEMIA
5 HEMOLYTIC ANAEMIA

ABSOLUTE CD4 COUNT

1 - >500 CELS/ µl
2 - 200-500 CELS/ µl
3 - <200 CELS/ µl

HIV RNA VIRAL LOAD

1 - <200 COPIES/ML

2 - 200-1000 COPIES/ML

3 - >1000 COPIES/ML

BONE MARROW PICTURE

1-Erythroid hyperplasia with mild dyserythropoiesis, megakaryocytic hyperplasia and mild increase in plasma cells

2- Immune thrombocytopenia